(19) **United States**
(12) **Patent Application Publication**
Rothenberg et al.
(10) **Pub. No.: US 2021/0080453 A1**
(43) **Pub. Date: Mar. 18, 2021**

(54) **BLOOD BIOMARKER FOR EOSINOPHILIC GASTROINTESTINAL DISORDERS**

(71) Applicant: **Children's Hospital Medical Center**, Cincinnati, OH (US)

(72) Inventors: **Marc E Rothenberg**, Cincinnati, OH (US); **Ting Wen**, Cincinnati, OH (US); **Yrina Rochman**, Cincinnati, OH (US)

(21) Appl. No.: **15/733,755**

(22) PCT Filed: **Apr. 18, 2019**

(86) PCT No.: **PCT/US2019/028076**

§ 371 (c)(1),
(2) Date: **Oct. 16, 2020**

Related U.S. Application Data

(60) Provisional application No. 62/660,308, filed on Apr. 20, 2018.

Publication Classification

(51) **Int. Cl.**
G01N 33/50 (2006.01)
G01N 33/68 (2006.01)

(52) **U.S. Cl.**
CPC ***G01N 33/505*** (2013.01); ***G01N 33/6893*** (2013.01); *G01N 2333/5437* (2013.01); *G01N 2333/5409* (2013.01); *G01N 2800/06* (2013.01)

(57) **ABSTRACT**

The disclosure provides a simple and rapid blood-based bioassay useful in the diagnosis, treatment and monitoring of eosinophilic gastrointestinal disorders, and related compositions and methods.

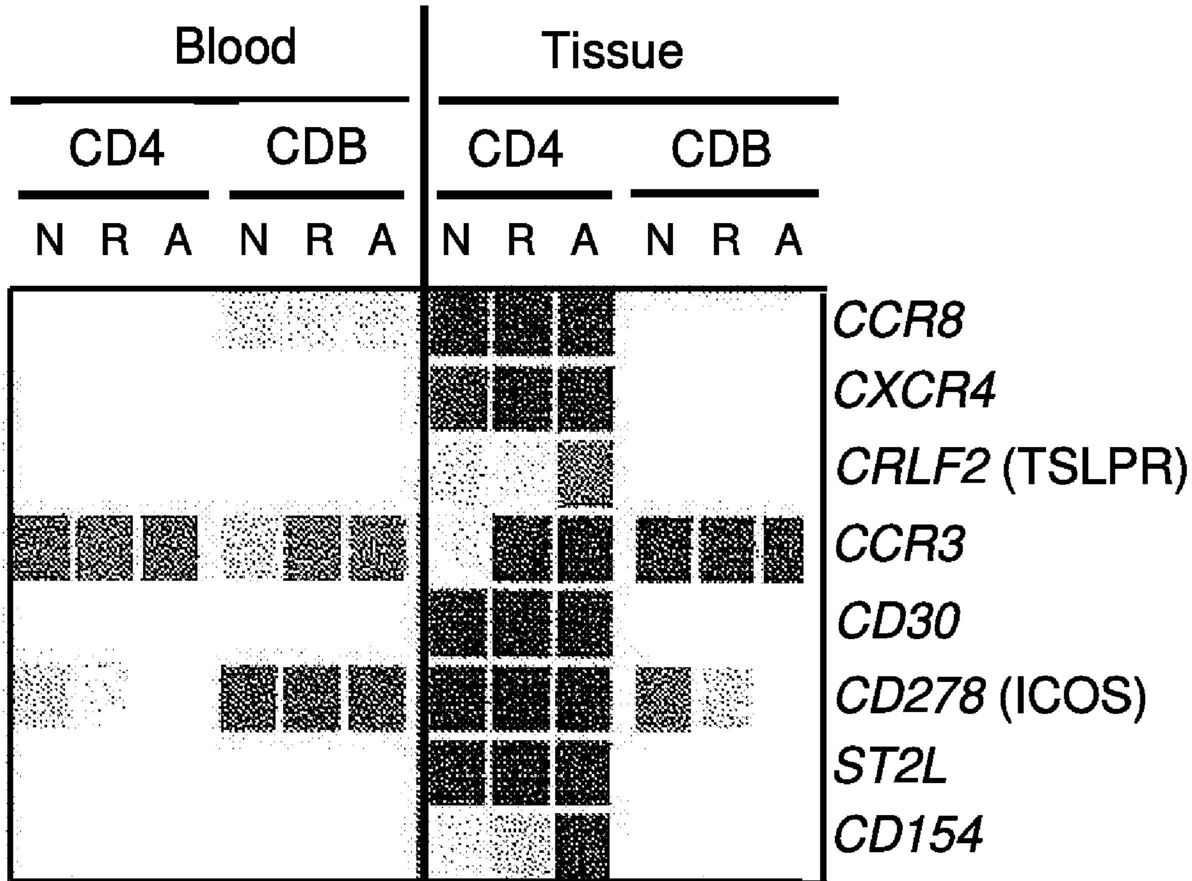


FIG. 1A

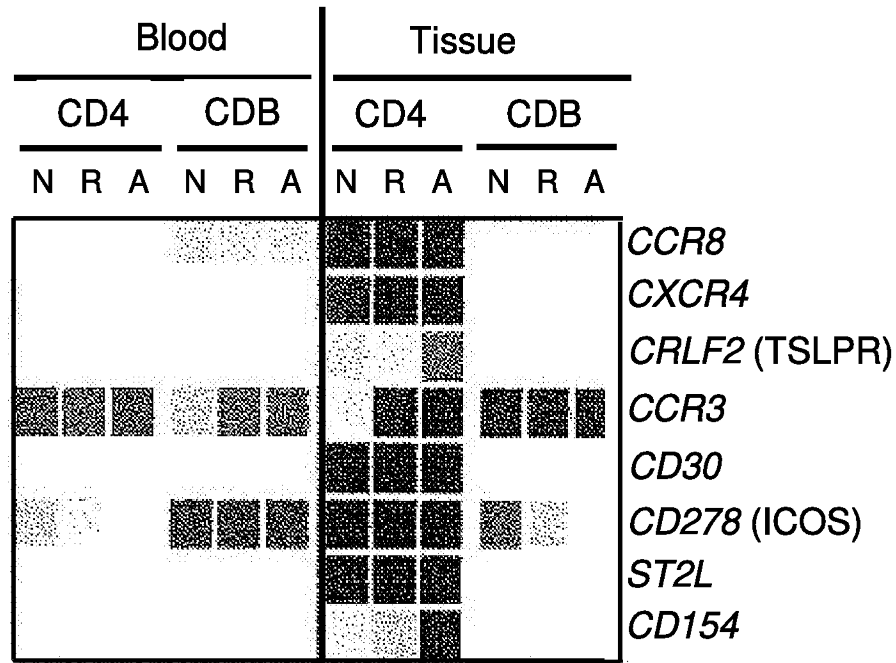


FIG. 1B

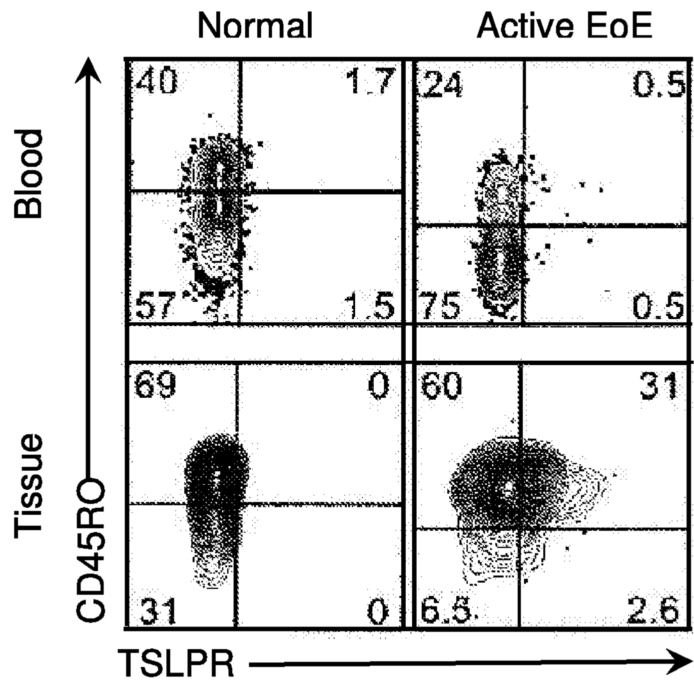


FIG. 2A

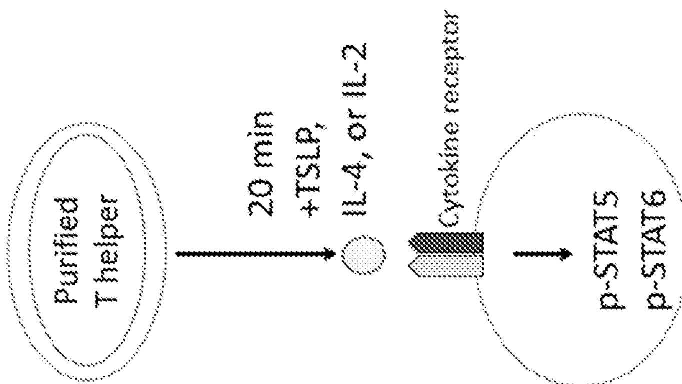


FIG. 2B

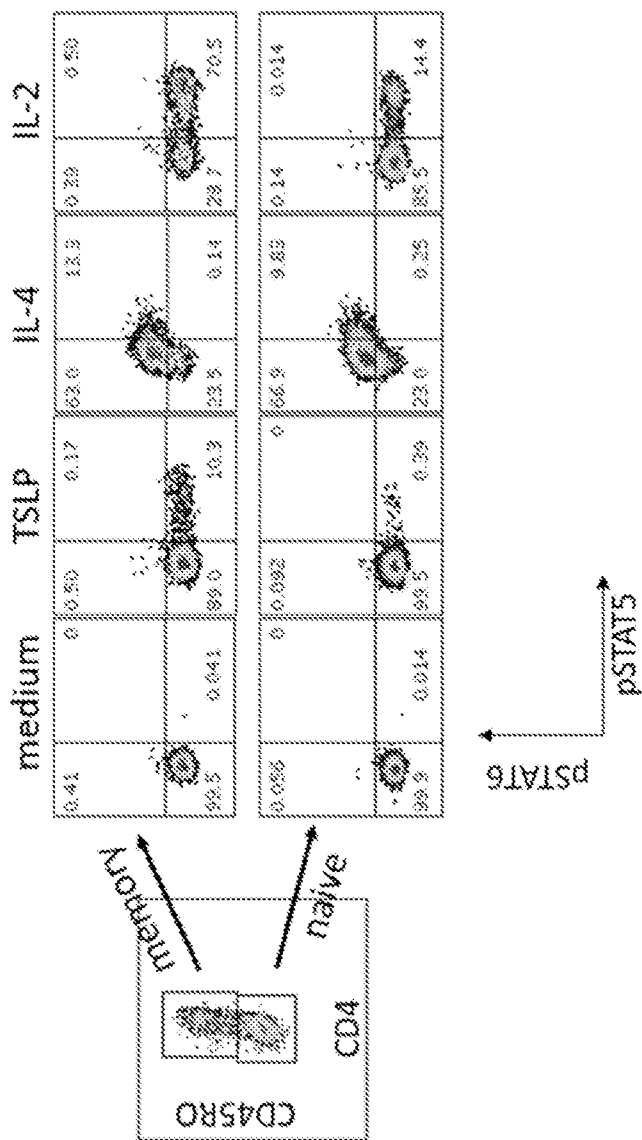


FIG. 2C

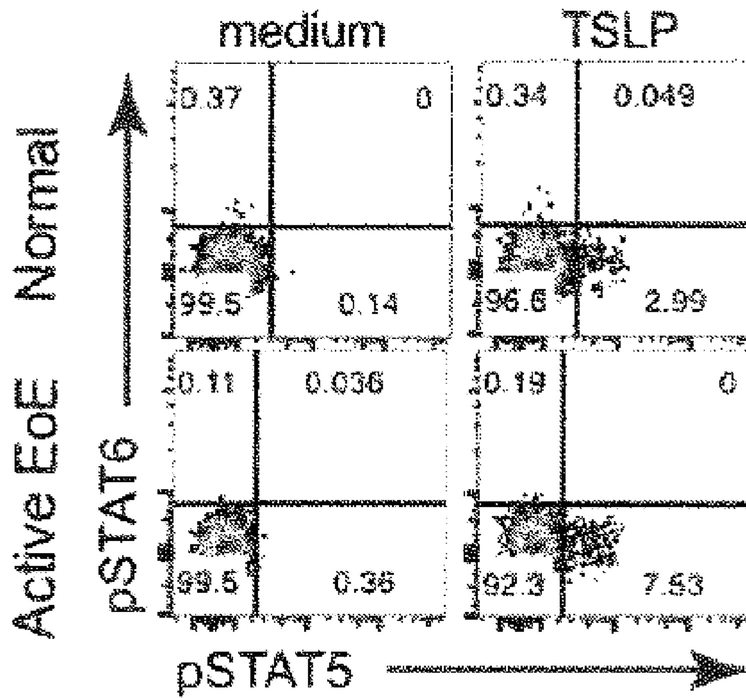


FIG. 3A

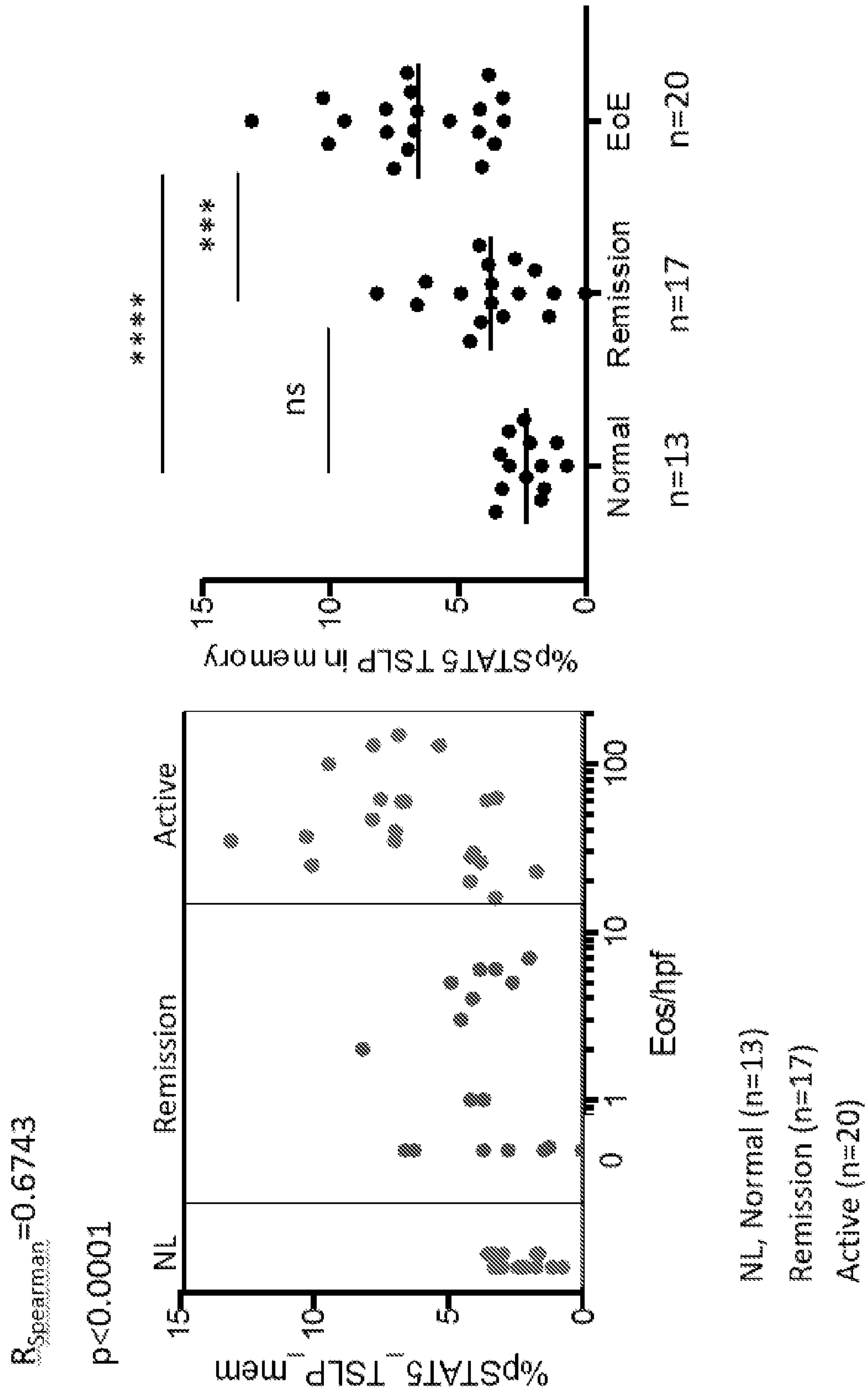
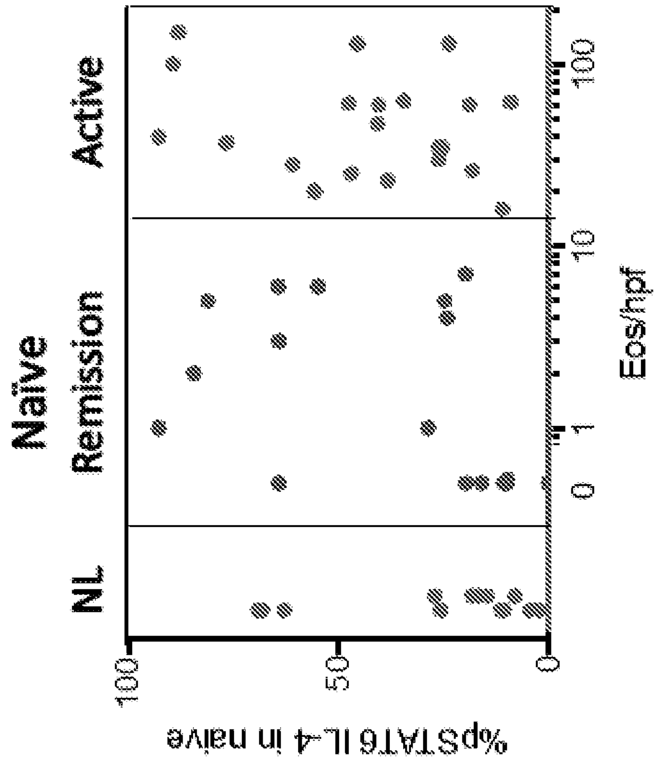


FIG. 3B

$R_{Spearman}=0.3426$
$P=0.0138$*



$R_{Spearman}=0.3668$
$P=0.0081$**

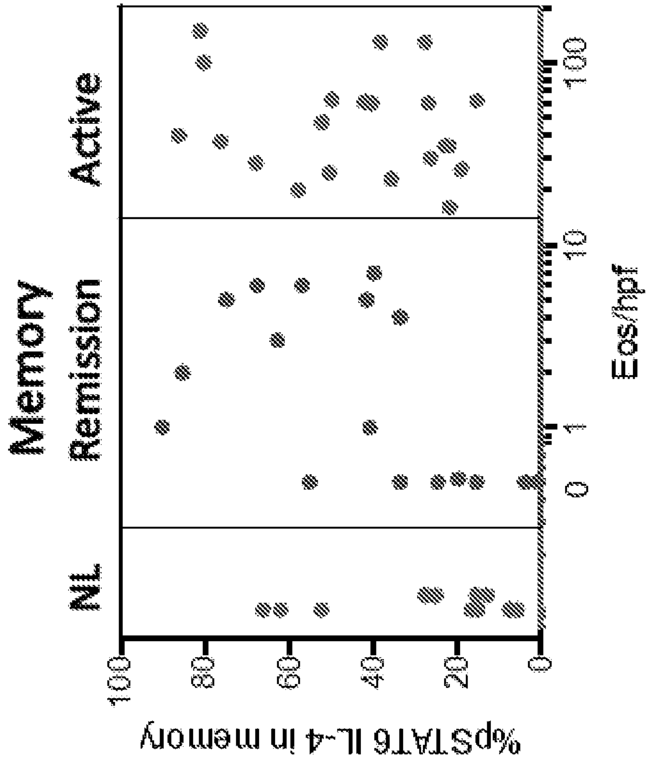


FIG. 3C

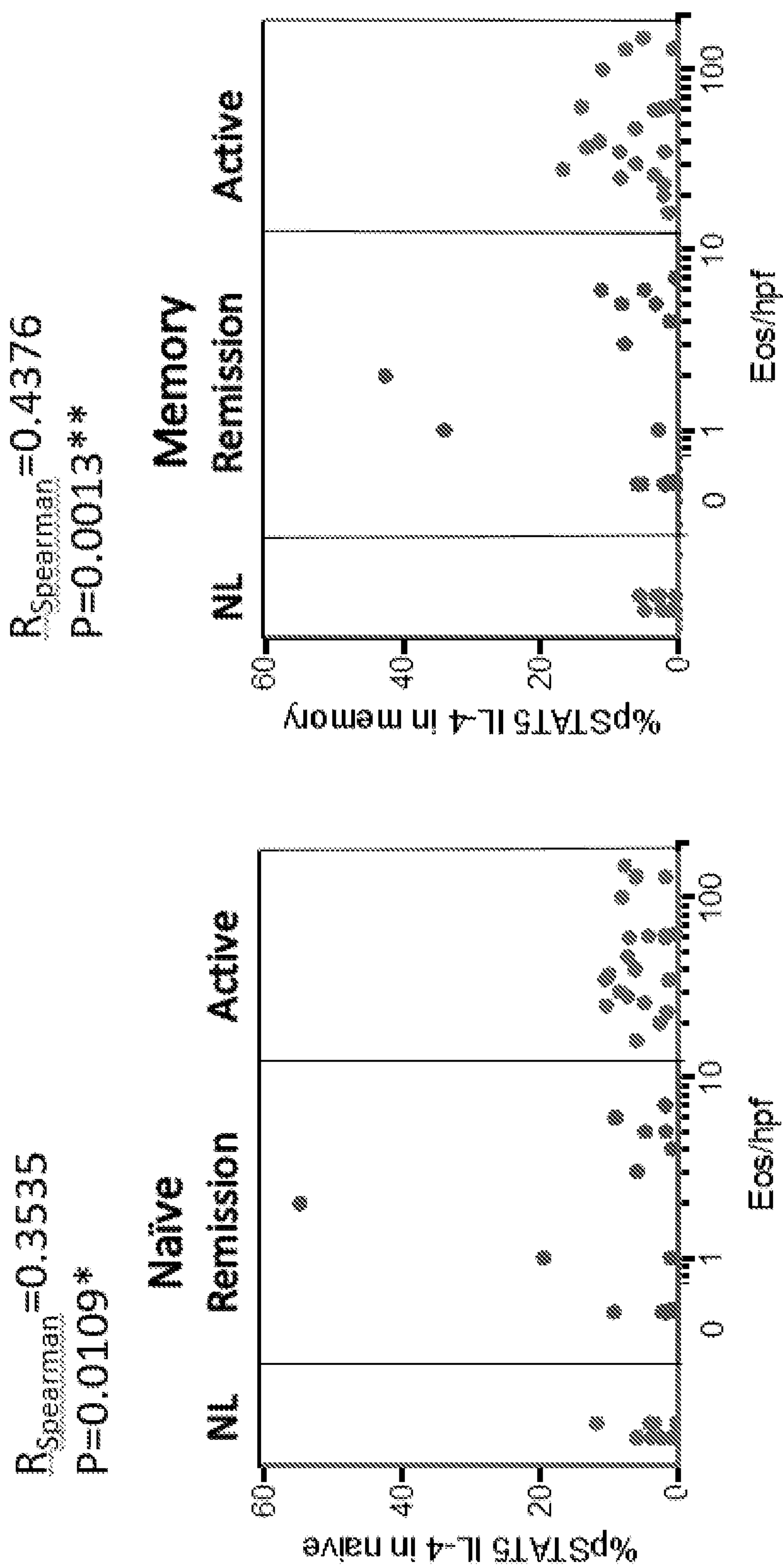


FIG. 3D

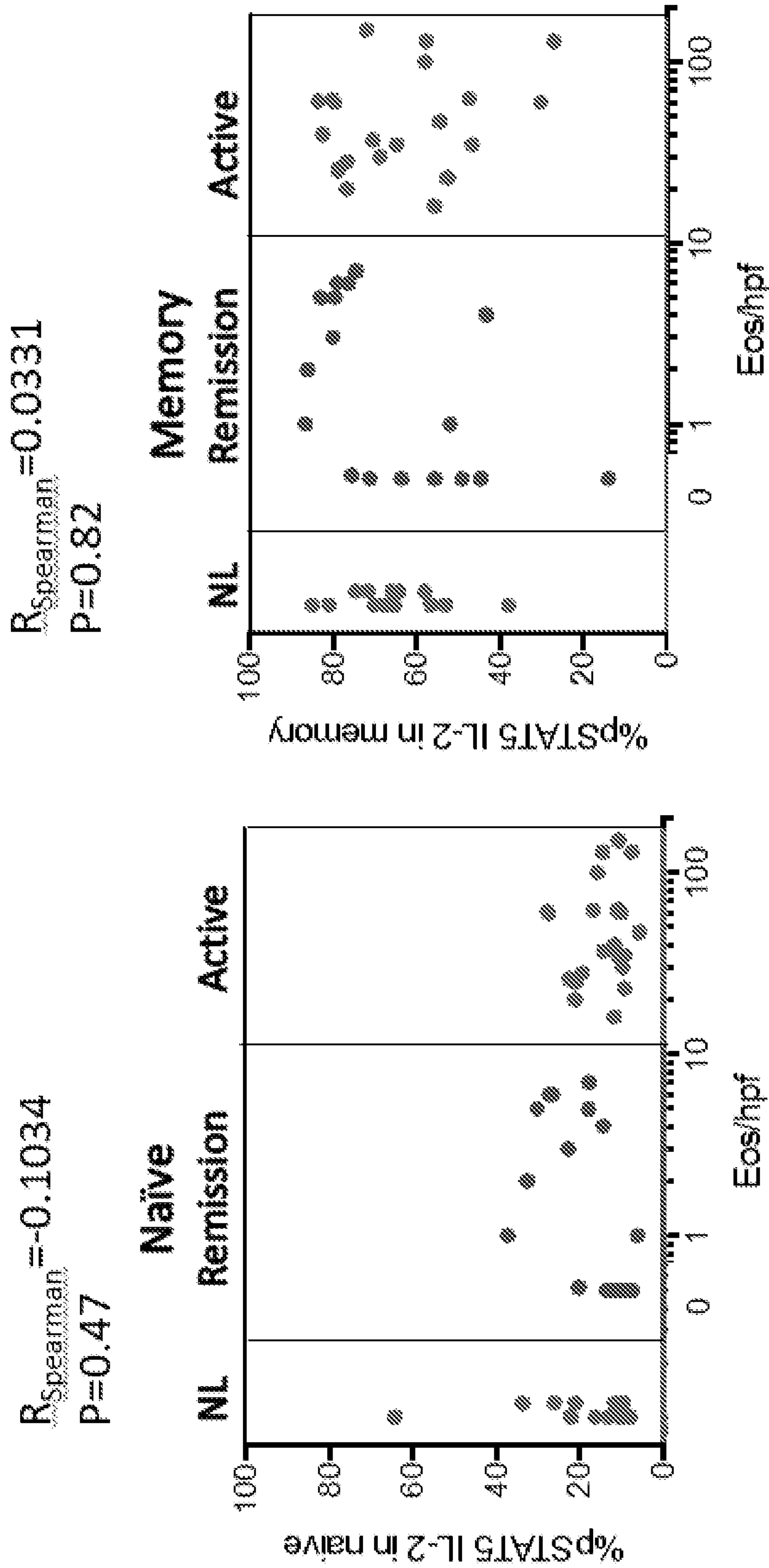


FIG. 4A

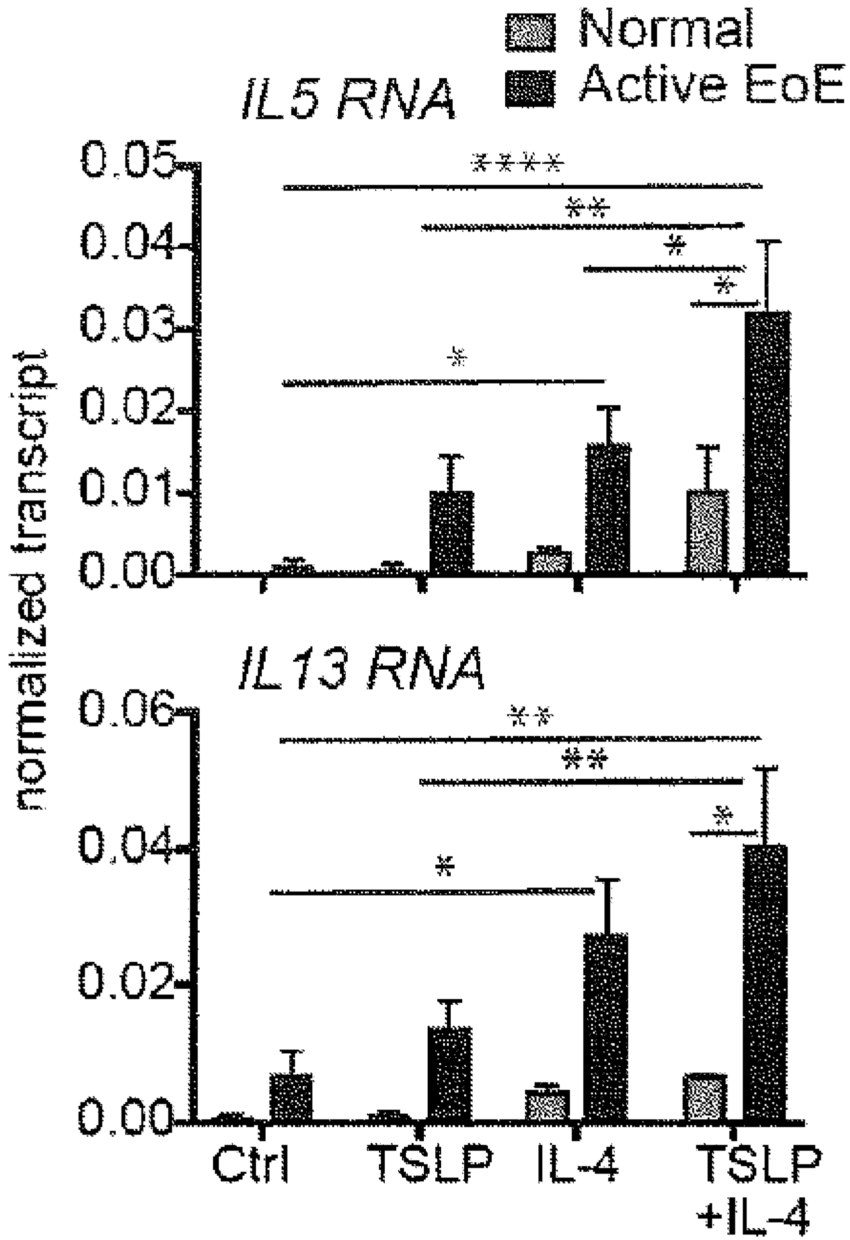


FIG. 4B

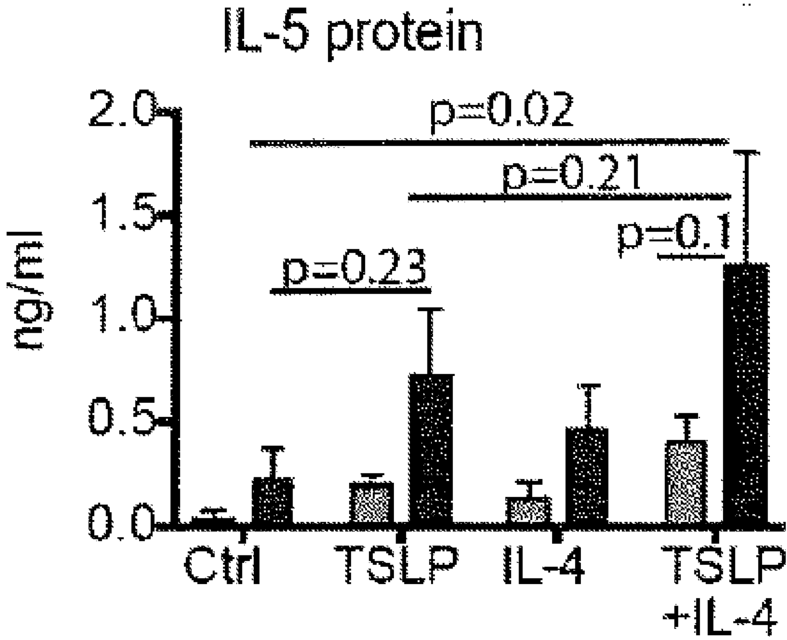


FIG. 5A

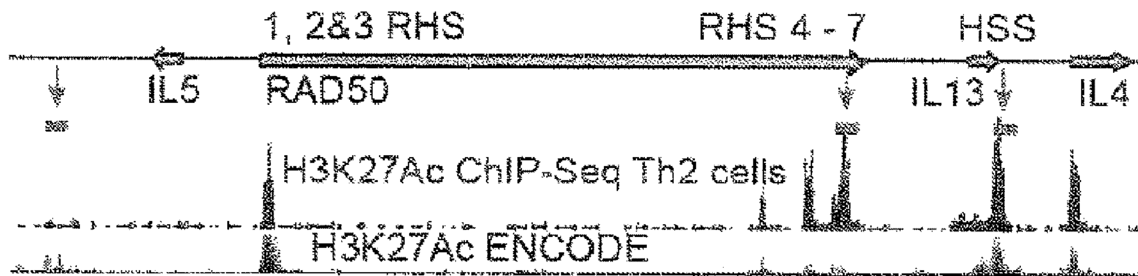


FIG. 5B

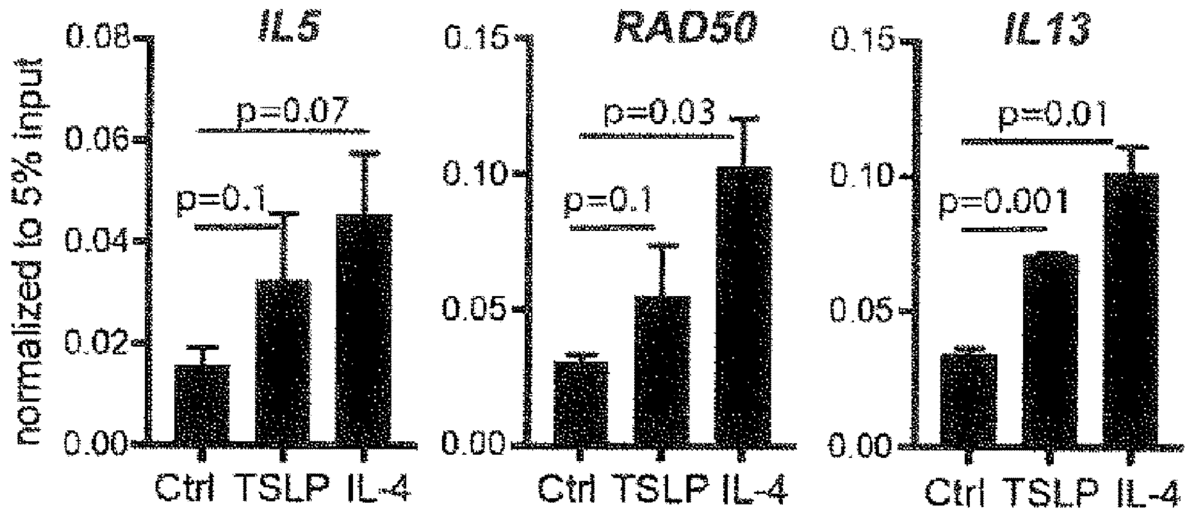


FIG. 6A

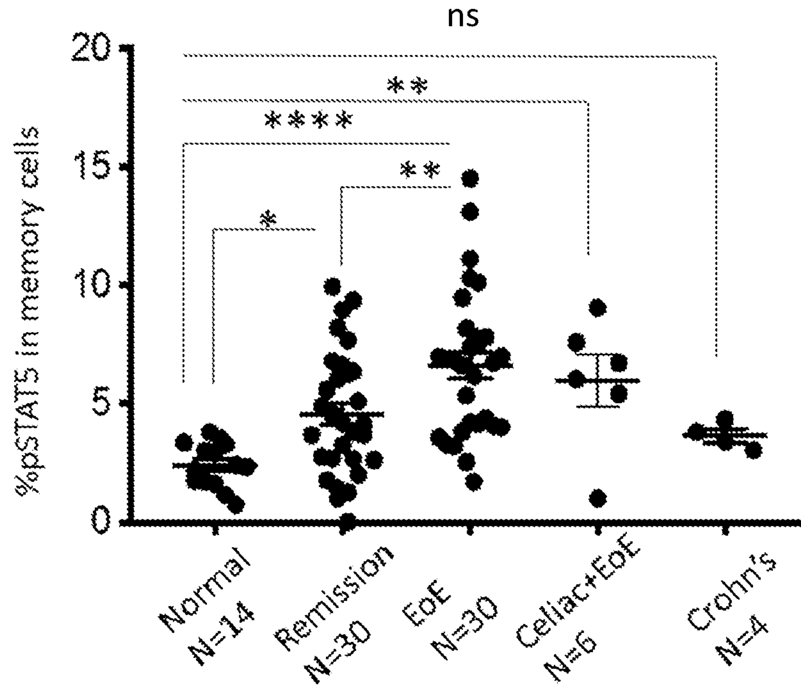


FIG. 6B

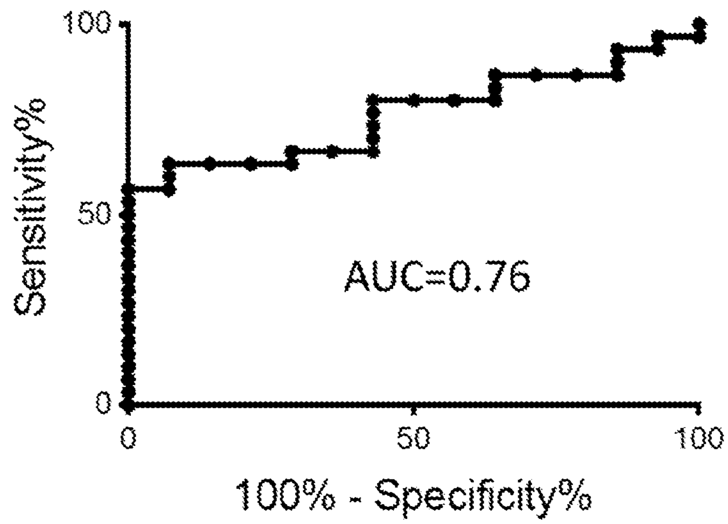


FIG. 6C

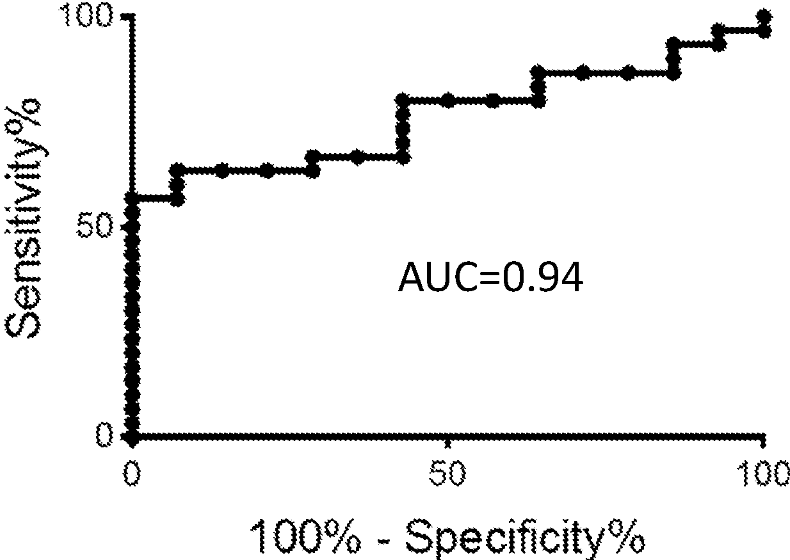


FIG. 6D

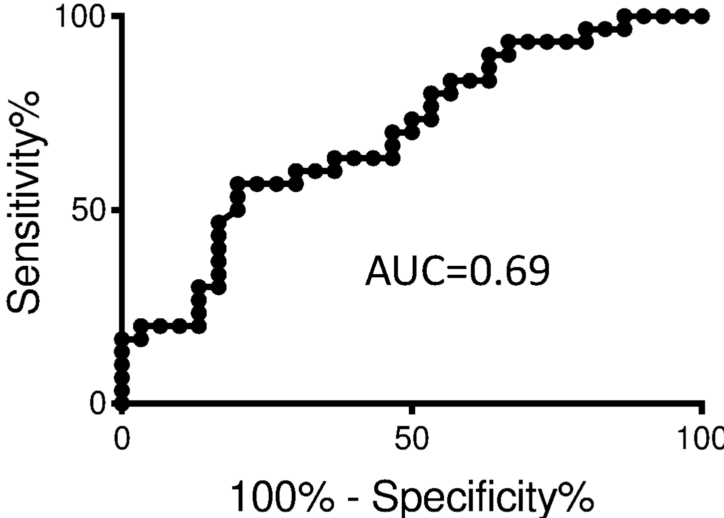


FIG. 6E

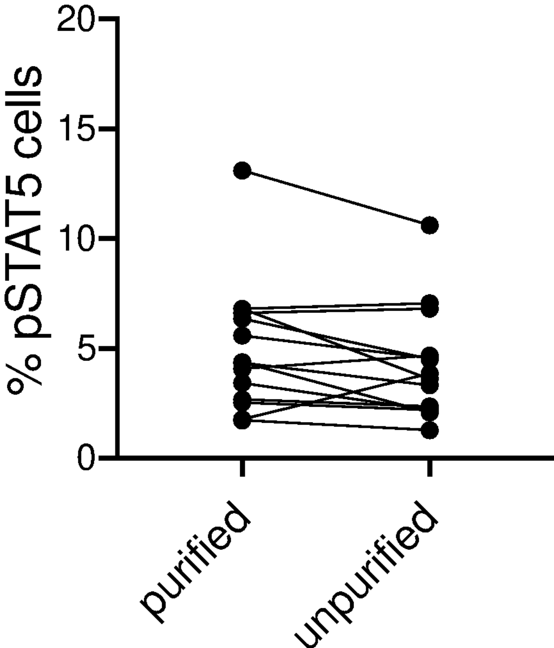


FIG. 6F

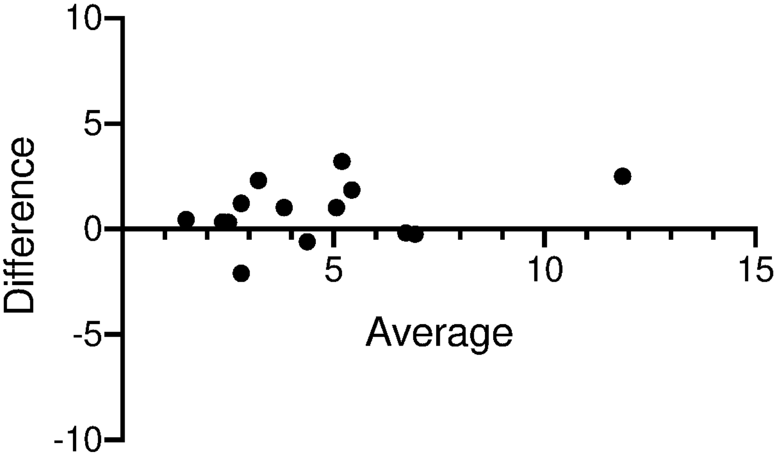


FIG. 7A

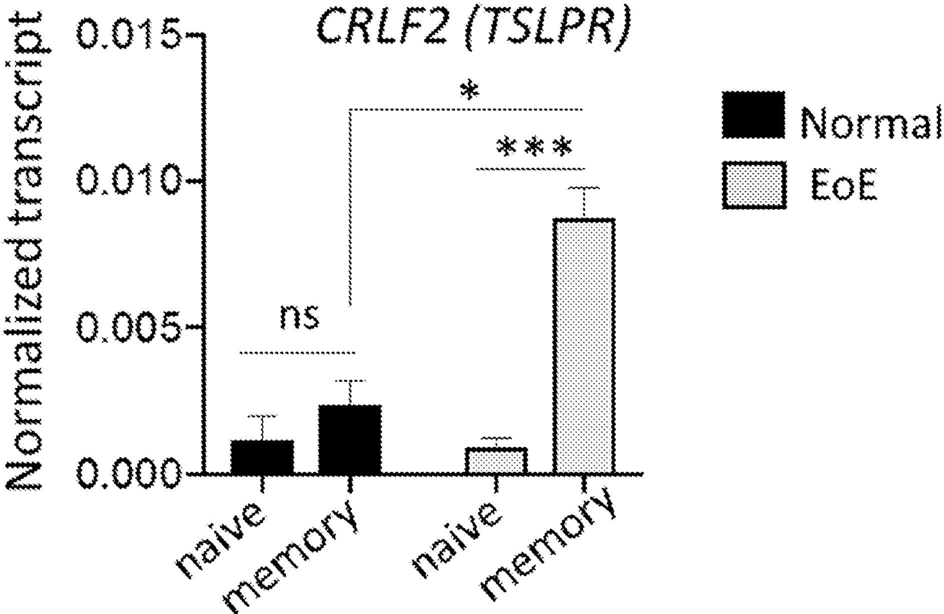


FIG. 7B

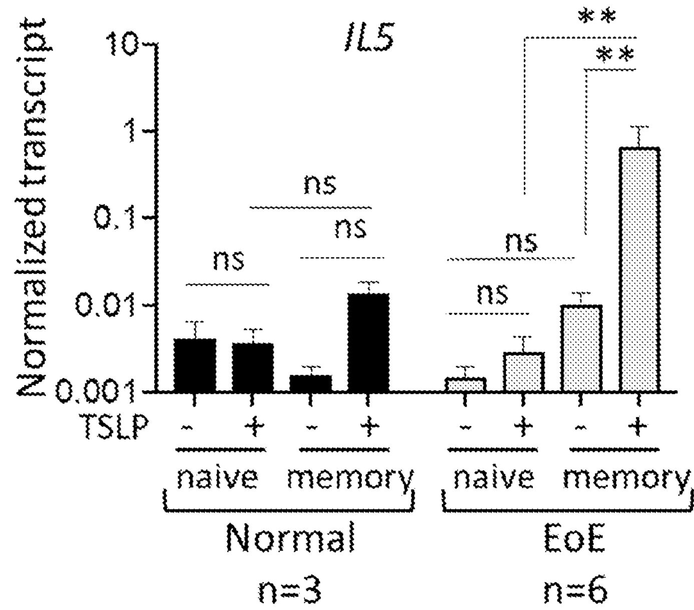


FIG. 7C

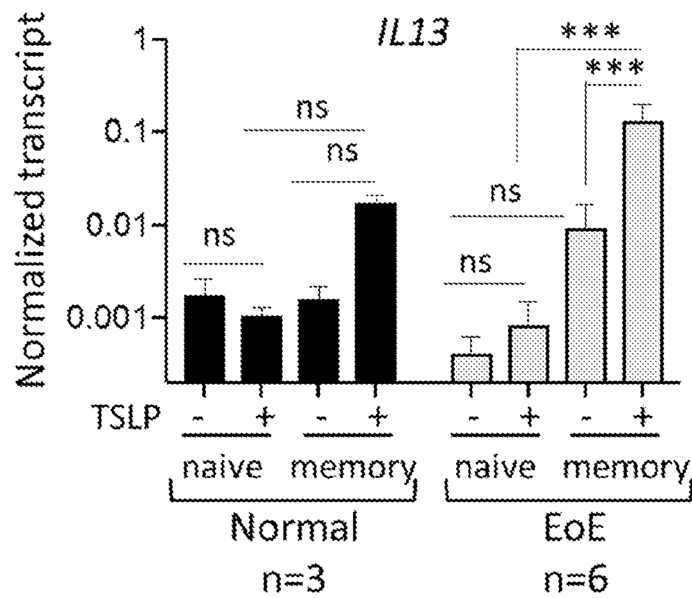


FIG. 7E

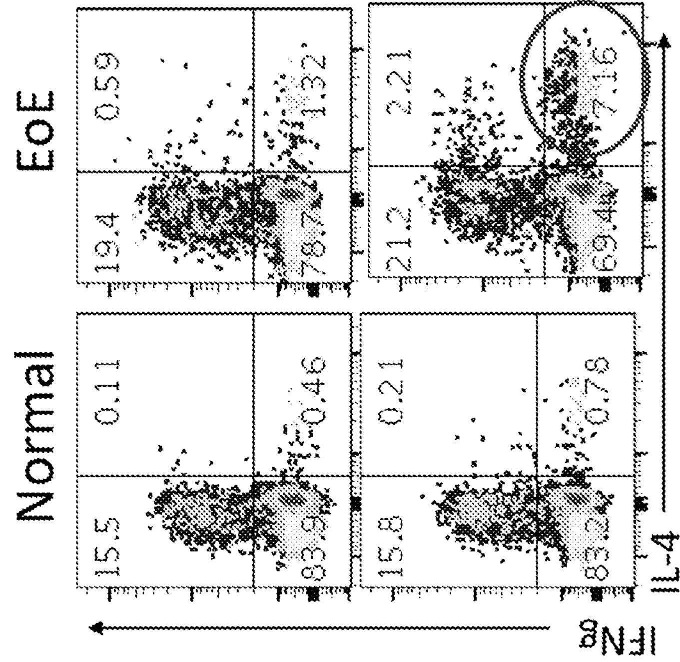


FIG. 7D

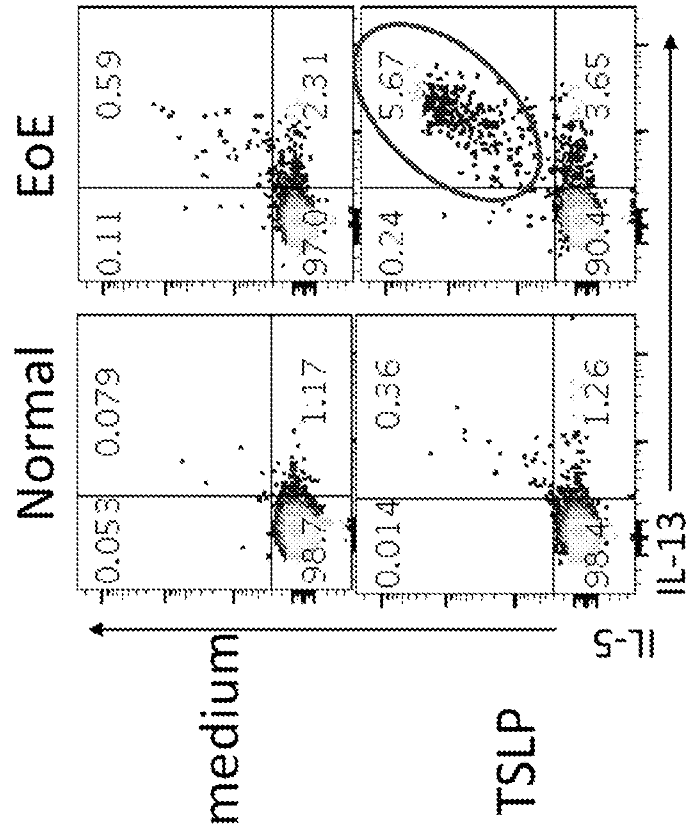


FIG. 8

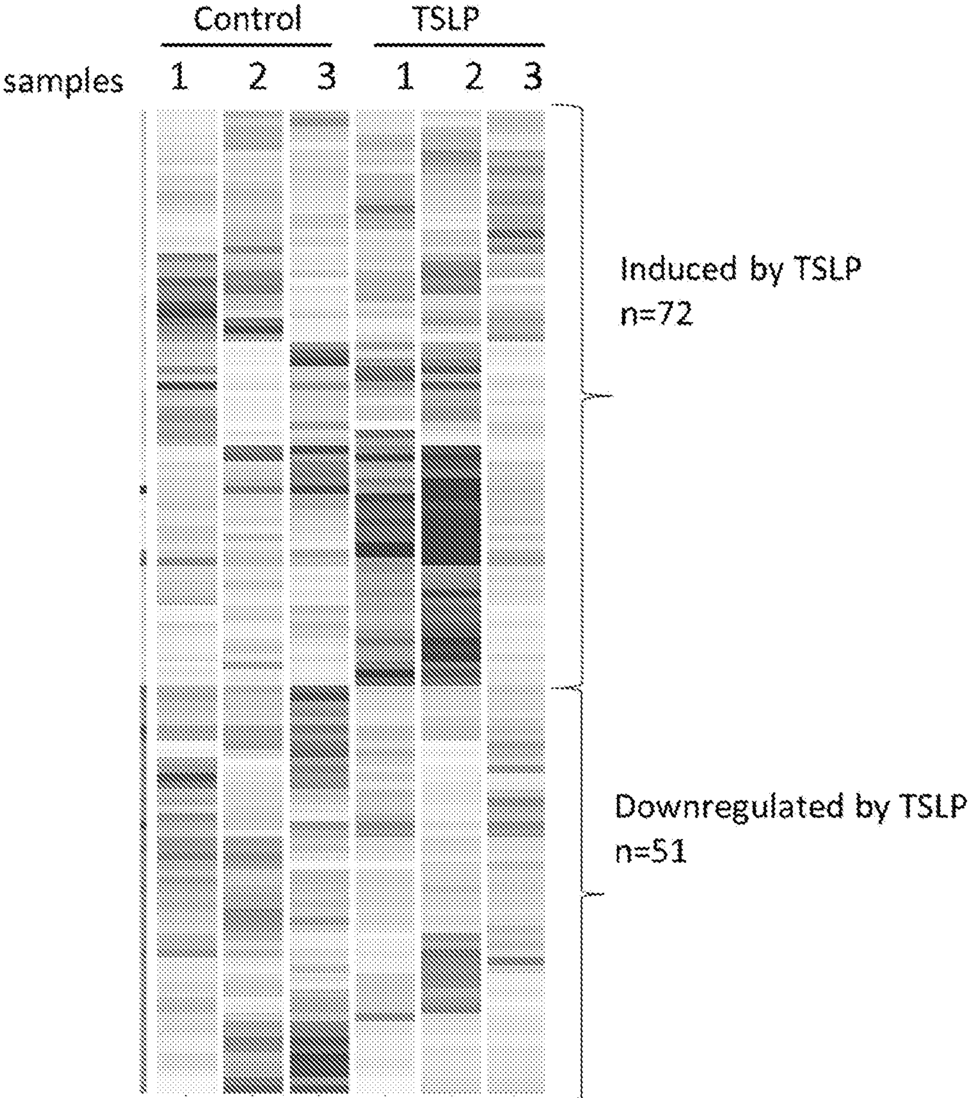
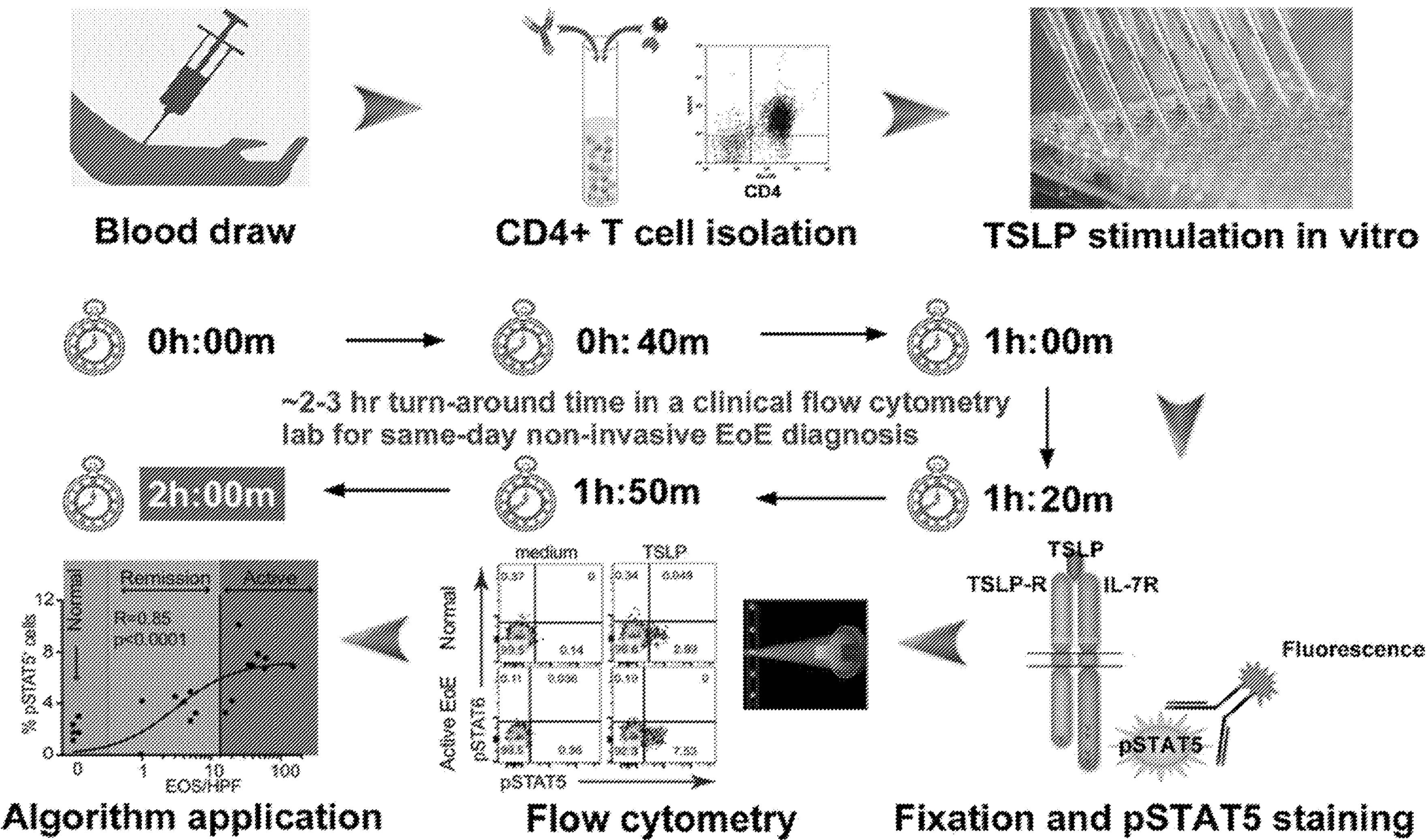


FIG. 9



BLOOD BIOMARKER FOR EOSINOPHILIC GASTROINTESTINAL DISORDERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/660,308, filed Apr. 20, 2018, the entire disclosure of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH

[0002] This invention was made with U.S. Government support under R01 AI 124355 awarded by the National Institutes of Health. The U.S. Government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The disclosure relates to methods for diagnosing, treating, and monitoring eosinophilic gastrointestinal disorders using a blood-based biomarker assay.

BACKGROUND OF THE INVENTION

[0004] The cytokine thymic stromal lymphopoietin (TSLP) is one of the major pro-inflammatory and pro-allergic cytokines in humans. It is expressed primarily by epithelial cells, especially in the lung and skin and has been linked with the initiation and progression of allergic inflammatory disease. For review of TSLP and allergic disease, see Ziegler, *J. Allergy Clin Immunol.* 2012 130:845.

[0005] Presently, a definitive diagnosis of eosinophilic gastrointestinal disorders (EGID) such as eosinophilic esophagitis (EoE) and eosinophilic gastritis (EG) depends upon a costly and uncomfortable endoscopic procedure followed by histologic examination of the tissue biopsy to assess the number of eosinophils per high power field, which remains the 'gold standard' of diagnosis. There is a need for improved assays to detect, diagnosis and monitor EGID to improve clinical care in human patients. The present disclosure addresses this need.

SUMMARY OF THE INVENTION

[0006] The present invention is based, in part, on the surprising discovery of a distinct subpopulation of memory CD4+T helper cells present in the peripheral blood of human patients with active eosinophilic gastrointestinal disorder (EGID) that is responsive to thymic stromal lymphopoietin (TSLP). Moreover, TSLP responsiveness, as determined according to the methods described here, correlates with disease severity, providing the basis for a blood-based assay for the care and management of EGID. Accordingly, the disclosure provides blood-based biomarker assays, and related compositions and methods, for the diagnosis, treatment, and monitoring of EGID in human subjects.

[0007] The disclosure provides methods for detecting TSLP-responsive cells in human blood, the methods comprising

[0008] contacting cells in vitro with TSLP, wherein the cells are previously isolated from a sample of whole blood obtained from a human subject in need of treatment for an eosinophilic gastrointestinal disorder (EGID), and

[0009] detecting one or more analytes in the cells or secreted by the cells, the one or more analytes selected from phosphorylated signal transducer and activator of transcription 5 (pSTAT5), interleukin-5 (IL-5), and interleukin-13 (IL-13),

[0010] wherein the detection of the one or more of the analytes indicates TSLP responsive cells in the blood of the human subject.

[0011] In embodiments, the detection of the one or more analytes is determined to be positive or negative based upon a predetermined threshold. In embodiments, a positive detection of the one or more analytes indicates TSLP responsive cells in the blood of the human subject.

[0012] In embodiments, the method for detecting TSLP-responsive cells in human blood is for use in a method for diagnosing or monitoring EGID in a human subject in need thereof.

[0013] In embodiments, the method further comprises determining an amount of the one or more analytes and determining whether the amount is above or below a diagnostic threshold, wherein an amount above the diagnostic threshold indicates an EGID status of active disease in a method for diagnosing, or disease progression in a method of monitoring EGID.

[0014] In embodiments, the cells are selected from peripheral blood mononuclear cells (PBMC), CD4+ T cells, and memory CD4+ T cells. In embodiments, the method further comprises a step of isolating a fraction of cells from the whole blood enriched for PBMC, CD4+ T cells, or memory CD4+ T cells.

[0015] In embodiments, the analyte is pSTAT5. In embodiments, the pSTAT5 is detected by a method comprising flow cytometry.

[0016] In embodiments, the analyte is IL-5 or IL-13 gene or protein expression. In embodiments, the IL-5 or IL-13 gene expression is detected by a method comprising a polymerase chain reaction (PCR), flow cytometry, or a chromatographic technique. In embodiments, the IL-5 or IL-13 protein expression is detected by a method comprising one or more of flow cytometry, immunoassay, and a chromatographic technique.

[0017] In embodiments, the EGID is eosinophilic esophagitis (EoE), eosinophilic gastritis (EG), or eosinophilic gastroenteritis (EGE).

[0018] In embodiments, the subject in need is characterized as presenting with clinical features selected from one or more of dysphagia, food impactions, vomiting, abdominal pain, refractory reflux symptoms, failure to thrive in young children, a diagnosis of an atopic allergic disorder, and a family member having an EGID diagnosis. In embodiments, the atopic allergic disorder is selected from asthma, atopic dermatitis, allergic rhinitis and allergic conjunctivitis.

[0019] In embodiments, the method further comprises a step of administering an EGID therapy to the subject in need. In embodiments, the EGID therapy is selected from proton pump inhibitor therapy, dietary therapy, anti-cytokine therapy, anti-ALOX15 therapy, anti-TSLP therapy, anti-eosinophil therapy, glucocorticoid therapy, and esophageal dilation. In embodiments, the EGID therapy comprises anti-TSLP therapy. In embodiments, the anti-TSLP therapy is an immunotherapy. In embodiments, the anti-TSLP therapy comprises anti-TSLP monoclonal antibody therapy.

[0020] The disclosure also provides a rapid assay for detecting a thymic stromal lymphopoietin (TSLP) respon-

sive population of cells in human blood, the method comprising contacting cells in vitro with TSLP, wherein the cells are previously isolated from a sample of whole blood obtained from a human subject in need of treatment for an eosinophilic gastrointestinal disorder (EGID), and detecting phosphorylated signal transducer and activator of transcription 5 (pSTAT5) in the cells, wherein the presence of pSTAT5 indicates a TSLP responsive population of cells in the blood of the human subject. In embodiments, the assay may be performed within about 1-6 hours, preferably within about 2-4 hours.

[0021] In embodiments, the rapid assay is performed by a method comprising flow cytometric analysis of pSTAT5. In embodiments, the cells are selected from PBMC, CD4+ T cells, or memory CD4+ T cells, preferably memory CD4+ T cells. In embodiments, the method further comprises isolating PBMC, CD4+ T cells, or memory CD4+ T cells from the sample of whole blood.

BRIEF DESCRIPTION OF THE FIGURES

[0022] FIGS. **1**A-B: A, flow cytometry analysis of $CD4^+$ and $CD8^{\pm}$-gated T cells from blood and biopsies of normal (N), EoE remission (R), and active EoE (A) patients to detect the expression of a panel of cytokine receptor genes. B, flow cytometric double plots of $CD3^+$ $CD4^+$-gated cells stained for TSLPR and CD45RO.

[0023] FIGS. **2**A-C: A, schematic of cytokine stimulation of human $CD4^+$ T cells isolated from blood. Cells were stimulated for 20 minutes with the indicated cytokine, TSLP, IL-2, or IL-4 and assayed for cytokine responsiveness. Responsiveness to IL-4 was assayed by detection of phosphorylated STAT6 (pSTAT6) and to IL-2 or TSLP by detection of phosphorylated STAT5 (pSTAT5). B, flow cytometry plots showing (left most panel) separation of naïve from memory CD4+ T cells via expression of CD45RO and the percentage of either memory (top four panels) or naïve (bottom four panels) CD4+ T cells detected as positive for either pSTAT6 or pSTAT5 under the indicated conditions of no cytokine stimulation (medium) or stimulation with each of TSLP, IL-4, or IL-2. C, flow cytometry plots showing the percentage of pSTAT5 and pSTAT6 positive memory CD4+ T cells in blood obtained from healthy donors (Normal) and patients with active EoE (Active EoE) following either no stimulation (medium) or in vitro stimulation with TSLP (TSLP).

[0024] FIG. **3**A-D: A, TSLP responsiveness measured as the percentage of pSTAT5 positive memory CD4+ T cells following in vitro stimulation with TSLP of cells obtained from the blood of healthy donors (Normal or NL), EoE patients in remission (Remission), or EoE patients with active disease (EoE or Active) positively correlates with the number of eosinophils per high power field (Eos/HPF) determined from tissue biopsies. In contrast, a poor correlation is seen with responsiveness to IL-4 as measured by the percentage of pSTAT6 positive (B) or pSTAT5 positive (C) naïve or memory CD4+ T cells following in vitro stimulation with IL-4. D, no correlation is observed between responsiveness to IL-2 as measured by the percentage of pSTAT5 positive naïve or memory CD4+ T cells following in vitro stimulation with IL-2.

[0025] FIG. **4**A-B: $CD4^+$ T cells were isolated from healthy subjects (normal, light bars) and patients with active EoE (Active EoE, dark bars) and either left unstimulated (Ctrl) or stimulated with either TSLP, IL-4, or both TSLP and IL-4 for 3 days then analyzed for (A) gene expression of IL-5 (top panel) and IL-13 (bottom panel) using RT-PCR to quantitate mRNA levels, or (B) IL-5 protein secretion using ELISA. Mean+/−SEM.

[0026] FIGS. **5**A-B: (A) PCR primer design for H3K27Ac-ChIP-PCR. Three sets of TaqMan primers (gray rectangles below arrows) were designed based on ChIP-sequence results and ENCODE data. (B) Purified $CD4^+$ T cells from normal donors were activated in the presence or absence of TSLP or IL-4 for 3 days and subjected to ChIP with anti-H3K27Ac. PCR products were normalized to their inputs (5%). Data represent duplicate experiments. Mean+/−SEM.

[0027] FIG. **6**A-F: A, percentage of pSTAT5 positive memory T cells as a percentage of total memory T cells following in vitro TSLP stimulation of cells isolated from the blood of healthy donors (Normal, n=14), EoE patients in remission (Remission, n=30), EoE patients with active EoE (EoE, n=30), patients with Celiac disease and active EoE (Celiac+EoE, n=6) and patients with Crohn's disease (Crohn's, n=4), which is a non-allergic autoimmune disorder (used as negative control); ROC curves showing sensitivity and specificity of the discrimination between B, normal and EoE remission; C, normal and active EoE; and D, remission and active EoE. E, Comparison of purified $CD4^+$ T cells and unpurified blood cells gated on CD4+ cell population in response to TSLP as percentage of pSTAT5 positive events inside of memory cell population (n=14 pairs). F, Bland-Altman plot shows differences in two methods (purified and unpurified $CD4^+$ T cells) measuring responsiveness of $CD4^+$ T cells to TSLP by STAT5 phosphorylation (n=14 pairs).

[0028] FIGS. **7**A-E: mRNA expression of A, CRLF2 (TSLPR), B, IL-5, and C, IL-13 in naïve and memory CD4+ T cells isolated from the blood of healthy donors (normal) and patients with active EoE (EoE) either without (−) or with (+) TSLP stimulation, B, C. Flow cytometry analysis of protein expression for IL-5 and IL-13 (D) or IFNg and IL-4 (E), either without (medium) or with (TSLP) TSLP stimulation.

[0029] FIG. **8**: Heat map showing individual results of expression profiling of memory CD4+ T cells obtained from 3 EoE patients, in the presence or absence of TSLP. Seventy-two transcripts were induced and fifty-one were downregulated by stimulation with TSLP compared to unstimulated.

[0030] FIG. **9**: Schematic depicting diagnostic assay process flow for rapid detection of TSLP responsive cells isolated from whole blood using a flow cytometry based assay.

DETAILED DESCRIPTION OF THE INVENTION

[0031] The inventors' discovery of TSLP-responsive memory CD4+T helper cells in the peripheral blood of human patients with active EGID, and the relationship to disease status, provides the basis for improved patient care in the form of a non-invasive blood-based assay for EGID status that can serve as an alternative or complement to endoscopy in the diagnosis and monitoring of EGID in human patients. The methods described here may advantageously form part of a therapeutic regimen effective to reduce the total number of endoscopies required to monitor disease and treat the EGID patient. In embodiments, the number of endoscopic procedures required may be reduced, e.g., from twice yearly to once per year or less.

[0032] Eosinophilic gastrointestinal disorders (EGID) are a diverse group of disorders characterized by increased eosinophil counts in one or more parts of the gastrointestinal tract in the absence of known causes for eosinophilia (e.g., secondary infections) or an underlying systemic inflammatory disease such as inflammatory bowel disease. EGID include disorders such as eosinophilic esophagitis (EoE), eosinophilic gastritis (EG), and eosinophilic gastroenteritis (EGE).

[0033] TSLP is one of the key pro-allergic cytokines associated with EGID, and in the context of EoE, a TSLP-elicited basophil response may contribute to pathogenesis. Noti et al., *Nature Med.* 2013 19:1005. Our laboratory previously reported elevated TSLP levels in the esophageal tissues of individuals with EoE. (Blanchard C., et al., *J. Clin. Invest.* 2006 116(2):536-547) and a strong association of single-nucleotide polymorphisms (SNPs) in the TSLP locus with susceptibility to EoE (Rothenberg M E., et al., *Nat. Genet.* 2010 42(4):289-291).

[0034] Although murine CD4+ T cells are known to respond to TSLP, freshly isolated human CD4+ T cells have little, if any, detectable TSLP receptor mRNA and are unresponsive to TSLP in vitro, although the cells do respond to IL-2 and IL-7 as evidenced by activation of signal transducer and activator of transcription 5 (STAT5), assayed by detection of phosphorylated STAT5 (pSTAT5) produced by the cells. Rochman I. et al., *J. Immunol.* 2007 178:6720-6724. The phosphorylation of STAT5 was previously identified as the immediate downstream signaling element induced by TSLP signaling through its receptor. Rochman et al., *Proc. Natl. Acad. Sci. USA* 2010 107:19455-19460. Isolated human CD4+ T cells can be "pre-activated" in vitro by stimulation with anti-CD3 and anti-CD28 antibodies which mediate TSLP receptor expression and induce cell responsiveness to in vitro stimulation with TSLP. Rochman et al., *J. Immunol.* 2007. However, prior to the current disclosure, it was not known that there were endogenous TSLP responsive cells present in the peripheral blood, or that these cells could be used as a biomarker of disease status in EGID.

Eosinophilic Gastrointestinal Disorders (EGID)

[0035] Although EGID are classified as "allergic disorders" they have distinct pathology compared to other allergic disorders such as asthma, atopic dermatitis, and celiac disease. The pathogenesis of EGID involves an immune/antigen driven Th2 response. $CD4^{+}$ T helper cells in the affected tissues produce increased amounts of pro-inflammatory Th2 cytokines including IL-4, IL-5, and IL-13, and these cells have been identified as the primary cellular sources of Th2 cytokines in the pathogenesis of EoE. Mitson-Salazar A., et al., *J. Allergy Clin. Immunol.* 2016; 137(3):907-918 e909; Wen et al., *J. Clin Invest.* 2019 Apr. 8; 130. pii: 125917. doi: 10.1172/JCI125917. In the context of the present disclosure, CD4+T helper cells may also be referred to interchangeably as "CD4+ T cells" or simply as "T helper cells", or "Th cells". Th2 cytokine transcripts and proteins have also been identified in EoE biopsy tissues and are implicated as key contributory factors in EGID, as evidenced by rodent EoE models and anti-IL-13 therapy in humans. Blanchard C., et al., *J. Allergy Clin. Immunol.* 2011 127(1):208-217; Hirano et al, *Gastroenterology* 2019 156 (3):592-603.

[0036] Standard diagnosis of EGID is dependent upon quantitative assessment of eosinophils in the affected tissue, e.g., esophageal tissue for EoE or gastric tissue for EG. For example, a peak eosinophil count of ≥15 intraepithelial eosinophils in one high-power field [HPF] is the benchmark for EoE diagnosis. Tissue for diagnosis is obtained using an expensive and uncomfortable invasive endoscopy procedure. Typically, following diagnosis and the initiation of therapy, a patient will need to undergo endoscopy at least twice a year in order to monitor disease progression and determine therapeutic efficacy. The present methods provide an alternative to endoscopy in the diagnosis and management of EGID in the form of a simple blood-based assay.

Blood-Based Bioassays for EGID

[0037] The blood-based bioassays described here represent a significant improvement in patient care because they reduce the need for repeated endoscopy, which is both expensive and uncomfortable. In accordance with the methods described here, TSLP-responsive cells are detected in a sample of whole blood obtained from a human subject in need of diagnosis, treatment or monitoring for EGID. Responsiveness to TSLP is determined using an in vitro assay in which isolated cells are stimulated with TSLP for a suitable period of time to induce TSLP-receptor mediated signal transduction. The isolated cells may be peripheral blood mononuclear cells (PBMC), CD4+ T cells, or memory CD4+ T cells. After TSLP stimulation, the cells are assayed for the activation or expression of one or more TSLP-targeted proteins selected from phosphorylated STAT5 (pSTAT5), interleukin-5 (IL-5), and interleukin-13 (IL-13). The inventors have determined that the level of TSLP-responsive cells determined in accordance with the methods described here is positively correlated with EGID disease status in human patients, enabling the use of TSLP-responsiveness as a biomarker in the diagnosis and management of EGID.

[0038] In embodiments, the methods comprise the detection of cells positive for pSTAT5, for example using a method suitable for the detection of specific phosphoproteins, including flourescence activated cell sorting, or "FACS", also referred to as "flow cytometry", chromatography based techniques, and solid or liquid phase immunoassays. Exemplary techniques include Western blotting, affinity chromatography, thin layer chromatography, high pressure liquid chromatography (HPLC), mass spectrophotometry coupled with HPLC (MS-HPLC), enzyme linked immunosorbent assay (ELISA), and immunohistochemistry (IHC). In some embodiments, combinations of these techniques may also be used.

[0039] In a preferred embodiment, pSTAT5 is detected using a flow cytometry based assay, using a labeled anti-phospho-Stat5 antibody comprising a detectable label suitable for detection by FACS analysis, e.g., a fluorescent label such as phycoerythrin (PE) or allophycocyanin (APC).

[0040] In embodiments, the disclosure provides a rapid flow-cytometry based assay for detecting TSLP-responsive cells in whole blood. The method is graphically depicted in FIG. **9**. The method can advantageously be performed in about 4-5 hours from the time of blood draw. The method comprises obtaining a sample of whole blood from the subject, isolating a CD4+ T cell fraction from the whole blood, culturing the CD4+ T cells in vitro and stimulating with TSLP to induce TSLP-dependent signal transduction,

fixing and staining the stimulated cells, detecting pSTAT5 in the cells using flow cytometry, and determining EGID disease status based upon the percentage of pSTAT5 positive cells detected.

[0041] In embodiments, the methods described here may also comprise the detection of IL-5 and IL-13 cytokine expression, for example by assaying for the secreted cytokines in the medium of in vitro cultured cells, or by assaying for cytokine gene or protein expression. Protein expression can be determined by various methods, including flow cytometry, chromatography based techniques and solid or liquid phase immunoassays, and combinations thereof, as described above. Gene expression can be determined, for example, using a polymerase chain reaction (PCR) based method, including a reverse transcription PCR reaction (RT-PCR), a flow cytometry based method, including microfluidics assisted fluorescence in situ hybridization (FISH), or a chromatographic technique such as Northern blotting.

[0042] The cells used in the assays for TSLP-induced cytokine activation and expression described here are obtained from a sample of whole blood obtained from a human subject in need of therapy for EGID, or who presents with clinical symptoms consistent with EGID, as described in more detail below. The term “whole blood” refers to a sample of blood containing cells, such as red blood cells (also referred to as erythrocytes), white blood cells, and platelets, as well as plasma, which is the liquid remaining after the cells are removed. In the context of the present methods, whole blood is obtained from a subject and assayed for the presence of TSLP-responsive CD4+ T cells. In some embodiments, the CD4+ T cells are memory T cells. In some embodiments, the CD4+ T cells are isolated from the peripheral blood mononuclear cell (PBMC) fraction of the whole blood. In other embodiments, the assays are performed using the PBMC fraction, without isolating the CD4+ T cells. The term “peripheral blood mononuclear cells” (PBMC) refers to the fraction of white blood cells in whole blood that contain a round nucleus and includes lymphocytes (T cells, B cells, natural killer cells) and monocytes. Other white blood cells, referred to generally as granulocytes, have multi-lobed nuclei and include neutrophils, basophils, and eosinophils.

[0043] In embodiments, the methods may comprise a step of obtaining whole blood from a subject, e.g., by phlebotomy. The blood collected is preferably venous blood which may be collected into a suitable container, e.g., a container comprising sodium heparin.

[0044] In embodiments, the methods may comprise a step of isolating a PBMC fraction from whole blood, for example by a method comprising density centrifugation, e.g., using Ficoll™ or similar reagent. The PBMC fraction, CD4+ T cells, or memory CD4+ T cells may also be isolated, for example, using a negative depletion or positive selection strategy, either in combination with density centrifugation or without a centrifugation step. In negative depletion, non-target cells are tagged with an affinity label which allows for their removal via an affinity reagent, such as an affinity column or similar device. Typically labeling for a negative depletion strategy is accomplished using labelled antibodies against a cell surface antigen located on the non-target cells, i.e., the cells which are to be depleted from the sample, thereby leaving a sample enriched in the target cell population. In positive selection, target cells are tagged with an affinity label and then separated from other non-target cells. Examples of suitable labels include magnetic beads and polyhistidine tags. In embodiments, a combination of one or more of density centrifugation, affinity chromatography, and flow cytometry, for example using an anti-CD4 receptor antibody and/or an anti-CD45 receptor antibody comprising a detectable label, may be used to isolate the PBMC fraction, the CD4+ T cell fraction, and/or the memory CD4+ T cell fraction from whole blood.

[0045] The methods described here comprise activating isolated cells in vitro with TSLP in order to induce TSLP-dependent signal transduction in the cells. Generally, the amount of TSLP used will be in the range of about 25-100 ng/ml in serum free medium. The time of activation with TSLP will vary depending on the nature of the downstream effector molecule to be detected. For example, where pSTAT5 is to be detected, very short time periods of TSLP stimulation are required, since activation of STAT5 via phosphorylation is an early event in TSLP-dependent signal transduction. In other case, longer periods of stimulation may be required, for example where cytokine gene expression is to be determined, or cytokine protein expression. The longest time periods are required where the analyte is secreted protein. In exemplary embodiments, the method comprises stimulating the cells with TSLP, e.g., about 25-100 ng/ml TSLP, for about 10-60 minutes, e.g., about 10, 20, 30, 45, or 60 minutes, preferably about 20 minutes for the detection of pSTAT5. The time period for mRNA and protein detections is 3-4 days.

[0046] In embodiments, the methods described here comprise determining whether the amount of one or more of the analytes, i.e., pSTAT5, IL-5, or IL-13, is above or below a predetermined diagnostic threshold, wherein the amount relative to the diagnostic threshold indicates EGID disease status, for example the absence of EGID, active EGID, or EGID in remission. In embodiments, an amount of analyte above the diagnostic threshold indicates active EGID in a method for diagnosing, or disease progression in a method of monitoring EGID. In embodiments, the analyte may be the number of CD4+ T cells positive for one or more of pSTAT5, IL-5 mRNA, or IL-13 mRNA. In embodiments, the analyte may be the amount of a cytokine produced by the CD4+ T cells, as measured by mRNA or protein expression, or secreted protein. The diagnostic threshold for each analyte is pre-determined based on the amount of the analyte in representative populations of healthy disease-free subjects and subjects in one or more disease categories, for example active EoE, EoE in remission, and Celiac disease with EoE. In embodiments, the performance of the method is measured using an area under the curve (AUC) receiver operating characteristics (ROC) curve.

[0047] In accordance with the methods related to EGID disease monitoring, a reduction in the amount of one or more analytes as described herein, i.e., pSTAT5, IL-5 or IL-13, compared to a baseline measurement for the patient, for example as determined at the start of therapy or before the initiation of therapy, or compared to a predetermined diagnostic threshold, indicates that the EGID is being managed by the current therapy. For example, a reduction in one or more of pSTAT5, IL-5 or IL-13 may be indicative of an absence of exposure to food allergens where the therapy comprises an elimination diet, or inhibition of Th2 cytokine production where the therapy comprises glucocorticoid treatment. Likewise, where the amount of one or more of the analytes increases relative to a baseline measurement or

predetermined diagnostic threshold, it indicates that the EGID therapy is not effectively managing the disease. In embodiments, the methods may further comprise adjusting a subject's EGID therapy based on the assay result.

[0048] In accordance with the methods described here, a human subject in need of treatment for an EGID may include one who has not yet been diagnosed with EGID but who presents with clinical symptoms of EGID. In embodiments, the subject in need presents with one or more clinical features selected from dysphagia, vomiting, food impaction, abdominal pain, refractory reflux symptoms, failure to thrive in young children, a diagnosis of an atopic allergic disorder, and a family member having an EGID diagnosis. In embodiments, the atopic allergic disorder is selected from food allergy, asthma, atopic dermatitis, allergic rhinitis and allergic conjunctivitis.

[0049] In addition, a human subject in need of treatment for an EGID may include one who has been diagnosed with EGID and is in need of disease monitoring, for example in order to evaluate the efficacy of an EGID therapy. In embodiments, the methods may also comprise a step of treating the subject diagnosed with EGID, and/or modifying the therapy of a subject undergoing monitoring, as described in more detail below.

[0050] In accordance with the methods described here, the subject may be treated with one or more EGID therapies, including, for example, proton pump inhibitor therapy, dietary therapy, anti-cytokine therapy, anti-ALOX15 therapy, anti-TSLP therapy, anti-eosinophil therapy, glucocorticoid therapy, and esophageal dilation. In the context of the present disclosure, the terms "treatment", "treating", or "treat" describe the management and care of a human subject for the purpose of combating EGID and may include the administration of a therapeutic agent as well as the administration of a therapy such as a restricted diet, including for example elemental and elimination diets, or a medical procedure such as esophageal dilation, to alleviate one or more symptoms or complications of EGID, such as EoE or EG, or to eliminate one or more symptoms or complications of EGID, thereby treating the EGID. Therapeutic agents may include small molecules, such as proton pump inhibitors and glucocorticoids, or biologic agents, such as therapeutic antibodies or nucleic acids, including interfering RNAs.

[0051] Proton pump inhibitor (PPI) therapy may include treatment with a PPI such as dexlansoprazole, esomeprazole, lansoprazole, omeprazole, pantoprazole, and rebeprazole.

[0052] Dietary therapy may include, for example, elemental and elimination diets.

[0053] Anti-cytokine therapy may include, for example, a biologic agent targeted to inhibit cytokine signaling by one or more cytokines via their cognate receptors. In embodiments, the anti-cytokine therapy is an anti-T helper type 2 (Th2) therapy. A Th2 immune response is generally characterized by the production of interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13). Accordingly, an anti-Th2 therapy encompasses a therapy targeting one or more of IL-4, IL-5, and IL-13, and/or their receptors in order to inhibit IL-4, IL-5, and/or IL-13 mediated signal transduction. The most common biologics for anti-cytokine therapy are antibodies, preferably monoclonal antibodies, and most preferably fully human or humanized monoclonal antibodies. In embodiments of the methods described here, the anti-cytokine therapy is an anti-T helper type 2 (Th2) therapy selected from one or more of a therapy targeting the IL-4 and/or IL-13 signaling pathway, and a therapy targeting the IL-5 signaling pathway.

[0054] Interleukin-4 and interleukin-13 both mediate inflammation through their receptors, with IL-13 also binding to type 2 IL-4 receptors. IL-4 and IL-13 signaling pathways thus overlap and therapies envisioned by the methods described here may target one or both of these signaling pathways. Therapies targeting IL-4 signaling include monoclonal antibodies such as dupilumab, which targets the IL-4 receptor alpha (IL-4Ra). Therapies targeting IL-13 signaling include monoclonal antibodies such as RPC4046 or tralokinumab, both of which target IL-13.

[0055] Interleukin-5 (IL-5, CD125) is an eosinophil growth, activation, and survival factor. Humanized anti-IL-5 antibodies have been shown to be effective in treating asthma patients with the severe eosinophilic form of the disease, as discussed in Rothenberg M E., Cell 2016; 165(3): 509. Therapies targeting the IL-5 signaling pathway include, for example, therapies targeting IL-5 and its receptor, also known as CD125. Such therapies include monoclonal antibodies such as mepolizumab and reslizumab, which target IL-5, and monoclonal antibodies such as benralizumab, which target the IL-5 receptor.

[0056] Anti-ALOX15 therapy is therapy directed at suppressing the expression or activity of the ALOX15 gene product, arachidonate 15-lipoxygenase. Examples of ALOX15 inhibitors include PD146176.

[0057] Anti-TSLP therapy may take the form of immunotherapy, for example using an antibody against TSLP, such as tezepelumab. Corren J., et al., *New Engl. J. Med.* 2017; 377(10):936-946.

[0058] Anti-TSLP therapy or anti-ALOX15 therapy may also comprise the administration of a single or double stranded ribonucleic acid (RNA) agent that inhibits the expression of the TSLP gene or the ALOX15 gene, for example, by catalyzing the post-transcriptional cleavage of the target mRNA, or by inhibiting transcription or translation of the target mRNA. In embodiments, the RNA agent is a double stranded or single stranded RNA interference-based agent (RNAi). The RNAi agent may be based on a microRNA (miRNA), a short hairpin RNA (shRNA), or a small interfering RNA (siRNA). The RNAi agent comprises a region that is at least partially, and in some embodiments fully, complementary to the target RNA. Although perfect complementarity is not required, the correspondence should be sufficient to enable the RNAi agent, or its cleavage product in the case of double stranded siRNA or RNAi agents comprising cleavable linkers, to direct sequence specific silencing of the target mRNA, e.g., by RNAi-directed cleavage of the target mRNA.

[0059] Glucocorticoid therapy may comprise, for example, therapy with one or more glucocorticoids selected from fluticasone, prednisone and budesonide.

[0060] The methods of the present disclosure are preferably applicable to human subjects, also referred to as "patients", but the methods may also be applied to other mammalian subjects. Accordingly, in embodiments a method described here may be performed on a "subject" which may include any mammal, for example a human, primate, mouse, rat, dog, cat, cow, horse, goat, camel, sheep or a pig. Preferably, the subject is a human. The term "patient" refers to a human subject.

Examples

Example 1: A TSLP Responsive Subpopulation of Human Memory CD4⁺ T Cells is Present in Human Blood of Patients with EGID

[0061] We sought to determine whether there was a blood and tissue specific T-cell phenotype in patients with active EoE that could be exploited for disease detection, diagnosis, treatment and monitoring. We first performed a polychromatic fluorescence activated cell sorting (FACS) analysis on CD4+ and CD8+ T cells isolated from normal (NL), EoE remission (R), and active EoE (A) esophageal tissue biopsies to detect the expression of a panel of cytokine receptor genes. For all experiments, 'normal' donors were defined as healthy individuals that did not have a history of EoE or other allergic disorders. As shown in FIG. **1**A, TSLP receptor (CRLF2/TSLPR) expression was enriched in $CD4^+$ T cells of esophageal tissue and this expression was elevated in CD4+ T cells of tissue from patients with active EoE compared to both normal tissue and tissue from patients in remission, but TSLP receptor expression was not detectable in CD4+ or CD8+ T cells isolated from blood. The flow cytometry double plot shows that TSLP receptor expression in $CD45RO^+$ T cells was significantly increased in the tissue biopsies of active EoE patients compared to normal tissue, but this increase was undetectable in $CD45RO^+$ T cells isolated from blood (FIG. **1**B).

[0062] The failure to detect a difference in TSLP receptor expression in cells isolated from blood may have been a result of its much lower expression in CD4+ T cells of the blood as compared to tissue resident CD4+ T cells. We hypothesized that a downstream effector molecule of the TSLP-induced signaling pathway might be expressed at high enough levels to be used as a surrogate for TSLP-responsiveness. Since the phosphorylation of STAT5 is an early event in the activation of TSLP-induced signaling, we sought to determine whether phosphorylated STAT5 (pSTAT5) could be used as a biomarker for TSLP response in CD4+ T cells isolated from blood. FIG. **2**A shows a schematic representation of the assay in which human $CD4^+$ T cells were purified from whole blood of healthy or EoE donors and then stimulated for 20 min with either TSLP, IL-4, or IL-2, the latter two cytokines serving as positive controls for responsiveness to IL-4 as indicated by pSTAT6 and responsiveness to IL-2, as indicated by pSTAT5. In addition, we used CD45RO to separate the CD4+ T cells into naïve ($CD45RO^-$) and memory ($CD45RO^+$) subpopulations. The responsiveness of CD4+ T cells to TSLP was measured as the percentage of pSTAT5 positive cells detected following either no treatment or stimulation with TSLP, IL-4, or IL-2 (FIG. **2**B). Surprisingly, we found that only the memory T cell subpopulation, and not the naïve T cells, responded to TSLP as measured by the percentage of pSTAT5 positive cells (compare top (memory) and bottom (naïve) rows of panels in FIG. **2**B). In contrast, both memory and naïve CD4+ T cells responded to both IL-4 ($pSTAT6^+$) and IL-2 ($pSTAT5^+$). This experiment demonstrated that we could detect TSLP responsive CD4+ T cells in human blood by stimulating the cells in vitro with TSLP and assaying for pSTAT5 positive cells, and that the responsive cells were the memory T cell subpopulation. We next evaluated whether there was a detectable difference in TSLP responsiveness in memory $CD4^+$ T cells isolated from the blood of subjects with active EoE compared to those from healthy (normal) donors. FIG. **2**C shows that there was a significant increase in the percentage of TSLP-responsive cells from patients with active EoE compared to those obtained from normal donors.

[0063] We next examined whether there was a correlation between the percentage of TSLP-responsive memory Th cells ($pSTAT5^+$ $CD45RO^+$ $CD4^+$) and tissue eosinophil levels (EOS/HPF), which is the standard benchmark for EoE diagnosis and the only EoE severity monitoring parameter currently available. Dellon E S., *Gastroenterol. Hepetol. NY* 2011 7(11):742-744. As shown in FIG. **3**A, the TSLP responsiveness of memory Th cells isolated from whole blood, as measured by percentage of pSTAT5 positive cells, was positively correlated with the number of eosinophils per high power field (EOS/HPF), as determined by analysis of esophageal tissue biopsies, in each of the categories of subject evaluated, healthy donors, EoE patients in remission, and patients with active EoE. Patients with active EoE, defined as having at least 15 EOS/HPF, exhibited a significant increase in the percentage of pSTAT5 positive cells compared to normal donors. Notably, patients in EoE remission, defined as having 2-14 EOS/HPF, showed a moderate elevation in percentage of pSTAT5 positive cells, indicating a non-normalized Th compartment in the disease remission state ($p<0.001$, remission vs. EoE). In contrast, there was only a poor correlation between EoE status as measured by EOS/HPF and the responsiveness of memory or naïve CD4+ T cells to IL-4 as measured by the percentage of pSTAT6 (FIG. **3**B) or pSTAT5 (FIG. **3**C) positive cells. Further, there was no correlation for IL-2 responsiveness, as measured by pSTAT5 positive cells (FIG. **3**D). Taken together, these results indicate the pSTAT5 provides a suitable biomarker for measuring TSLP-responsive cells obtained from whole blood, and that there is a strong correlation between this biomarker and EGID disease status, making it an attractive candidate for a clinical assay.

[0064] In further experiments, we confirmed that the cells characterized as TSLP-responsive based on pSTAT5 also elicit de novo synthesis of Th2 cytokines IL-5 and IL-13, evidencing that the in vitro TSLP response observed in our assays represents a true activation of the proinflammatory pathway regulated by TSLP. The results of this study are shown in FIG. **4**. CD4+Th cells were isolated from the blood of normal healthy (NL) donors and patients with active EoE. Cells were cultured in vitro and activated by exposure to anti-CD3 and anti-CD28 antibodies and IFNγ depletion in the presence of IL-4 and/or TSLP for 3 days, then assayed for the expression of IL5 and IL13 mRNA (FIG. **4**A) and IL-5 protein (FIG. **4**B). Both TSLP and IL-4 induced the expression of IL5 and IL13 mRNA in CD4+Th cells isolated from patients with active EoE, but not in cells isolated from normal donors. Co-stimulation with both TSLP and IL-4 further increased the expression of these cytokines in the cells from active EoE patients, but only mild expression of these cytokines was detected in cells from healthy donors. IL-5 protein expression was also differentially increased in the CD4+Th cells isolated from patients with active EoE, compared to those isolated from normal donors.

[0065] We next examined whether the upregulation of Th2 cytokine expression correlated with epigenetic changes in the chromatin status of the cells, which would be a further indication that the response functions to activate TSLP receptor mediated signal transduction. CD4+Th cells were isolated from normal donors and activated in vitro as

described above. Cells were collected and fixed on day 3, followed by chromatin immunoprecipitation (ChIP) coupled with quantitative polymerase chain reaction (PCR) or "ChIP-PCR" analysis for detection of the histone mark H3K27Ac, which indicates areas of active chromatin. The data showed enhanced H3K27Ac downstream of the IL5 gene, in a locus control region in the RAD50 gene (RHS7), and in a hypersensitivity site (HSS) of an IL3 enhancer induced by TSLP (FIG. **5**A-B). These results are consistent with previously reported epigenetic signatures of Th2 cytokine loci. Similar chromatin modifications were observed in murine Th2 cells upon TSLP treatment, suggesting an evolutionarily conserved mechanism regulated by TSLP. H3K27Ac changes also correlated with transcription of the IL5 and IL13 genes in the presence of TSLP. Since these changes were observed with in vitro activated $CD4^+$ T cells from normal donors, which we have already determined contain a low percentage of TSLP-responsive cells, we expect a more robust signal of epigenetic modification will be observable in cells from patients with active EoE, making an assay based on chromatin immunoprecipitation coupled with RNA sequencing (ChIP-RNAseq) a highly feasible molecular diagnostic, genome-wide tool for eosinophilic gastrointestinal disease.

Example 2: Blood-Based Diagnostic Assay for EGID

[0066] The results described above demonstrate a robust positive correlation between the 'gold standard' diagnostic criterion of disease status, i.e., EOS/HPF in tissue biopsies, and the percentage of pSTAT5 positive cells following in vitro stimulation with TSLP using autologous blood memory $CD4^+$ T cells. This blood-based pSTAT5 assay therefore represents a promising non-invasive alternative to the conventional endoscopic method for detection, diagnosis, and monitoring of EGID.

[0067] In order to further validate this method for clinical use, we undertook a larger-scale study. Subjects were divided into three groups: healthy donors, designated as normal (NL); patients with EoE who were in remission; and patients with active EoE. In addition, blood samples were obtained from patients having both Celiac disease and EoE (Celiac+EoE, n=6) and patients with Crohn's disease (Crohn's, n=4), which is a non-allergic autoimmune disorder, as a negative control. TSLP-responsiveness determined as the percentage of memory CD4+ T cells that were also positive for pSTAT5 following in vitro stimulation with TSLP was determined as described above.

[0068] As shown in FIG. **6**A, the percentage of memory CD4+ T cells positive for phosphorylated STAT5 (pSTAT5) following in vitro TSLP stimulation was significantly different between (i) healthy donors and EoE patients in remission (*p=0.013), (ii) healthy donors and Celiac patients with EoE (**p=0.0063), (iii) health donors and patients with active EoE (**** p<0.0001)), and (iv) active EoE and EoE patients in remission (**p=0.0056). Patients with Crohn's disease had very low numbers of cells responding to TSLP, similar to the numbers for normal donors. In contrast, patients having both Celiac disease and EoE showed a significantly higher percentage of pSTAT5 positive cells, as did patients with active EoE and EoE patients in remission. These findings demonstrated a specific response of $CD4^+$ T cells to TSLP in patients with eosinophilic gastrointestinal disorders (EGID) as opposed to gastrointestinal autoimmune disorders, such as Crohn's disease. The clinical utility of these results was further validated by receiver operating characteristic (ROC) analysis. Memory $CD4^+$ T cells from EoE patients showed a strong increase in responsiveness to TSLP in the ROC curve data for normal vs. remission (FIG. **6**B), as well as for normal (n=14) vs. active EoE (FIG. **6**C) and EoE remission vs. EoE active (FIG. **6**D). Alternatively, the method without $CD4^+$ T cell isolation was used to detect pSTAT5 induced by TSLP. In this method, PBMCs, which contain variety of cell populations, were stimulated with TSLP for 20 minutes and then subjected to flow cytometry analysis. $CD4^+$ T cells were detected by gating strategy during flow cytometry analysis. In both methods, purified and unpurified $CD4^+$ T cells, blood samples from same donors were used and percent of pSTAT5 positive cells induced by TSLP was compared. Non-significant differences within the group were observed (FIG. **6**E-F). Therefore, unpurified $CD4^+$ T cells method could potentially be used. This will potentially further shorten the processing time and reduce the human error generated during the $CD4^+$ purification.

[0069] We further validated the biological significance of the observed TSLP responsiveness of $CD4^+$ T cells using a transcription assay to evaluate the gene expression of the TSLP receptor (CRLF2) and the pro-allergic cytokines IL-5, and IL-13. $CD4^+$ Th cells were isolated from the blood of normal and EoE patients and sorted using flow cytometry to differentiate between memory ($CD45RO^+$) and naïve ($CD45RA^-$) CD4+T subpopulations. The expression level of CRLF2/TSLPR mRNA was significantly higher within population of memory cells from EoE individuals compared to naïve cells and memory cells from normal donors (FIG. **7**A). The cells were then activated for 3 days in the presence or absence of TSLP. As shown in FIG. **7**B-C, memory $CD4^+$ Th cells from patients with active EoE expressed elevated levels of mRNA for each of IL5 (FIG. **7**B) and IL13 (FIG. **7**C). In addition, the increase in cytokine transcript expression was followed by an increase in protein production of cytokines IL-5 and IL-13 (FIG. **7**D) and IL-4, but not IFNγ (FIG. **7**E).

[0070] Overall, our results show that TSLP receptor expression is upregulated in $CD4^+$ T cells obtained from the blood of patients with active EoE at both the mRNA and protein levels and that TSLP strongly induces both pSTAT5 and the expression of Th2 cytokines downstream of STAT5 activation, IL-5 and IL-13 in these cells.

Example 3: TSLP-Induced Alterations in T-Cell Transcriptomes

[0071] To substantiate the finding that TSLP is a Th2 inducer in CD4+ T cells in the blood, we assessed the transcriptional profile of cytokines and other molecules induced by TSLP. To characterize the TSLP-induced transcriptomic changes in an EGID disease context, bulk RNAseq analyses of human blood memory CD4+ T cells was performed using blood obtained from three active EoE patients. In brief, the PBMC fraction was isolated from whole blood (5-10 mL) and the memory CD4+ T cell fraction was isolated and activated in vitro as described above, i.e., by exposure to anti-CD3 and anti-CD28 antibodies and IFNγ depletion in the absence or presence of TSLP for 3 days. Samples were subjected to bulk RNA sequencing and bioinformatics analyses using GeneSpring software.

[0072] The results of RNA sequencing are shown in FIG. **8** as a heat map. Samples 1-3 represent memory CD4+ T cells obtained from patients with active EoE. The first three rows show gene expression in unstimulated cells (control) and the next three rows show gene expression in cells stimulated with TSLP (TSLP). In the TSLP-activated memory CD4+ T cells derived from EoE active donors, TSLP induced 72 genes and downregulated 51 genes compared to controls. Many of the upregulated genes are known to be involved in allergic inflammation, which validates the importance of TSLP in initiation of allergic responses.

Example 4: Blood-Based pSTAT5 Assay for EGID

[0073] The results presented here indicate that the presence of TSLP-responsive memory T helper cells in peripheral blood can be used as an indicator of EGID status. Although the work presented here was performed in the context of EoE, we believe it is equally applicable to eosinophilic gastritis (EG) and eosinophilic gastroenteritis (EGE) because the majority of EG/EGE patients also have EoE and these EGID involve a similar type 2 allergic response.

[0074] A schematic depicting an exemplary embodiment of a rapid assay for detection of TSLP responsive cells isolated from whole blood is shown in FIG. **9**. The entire process from obtaining a blood sample from a subject to data output and EoE status determination may be performed in about 4-5 hours, including the time for initial isolation of the PBMC fraction from whole blood before CD4+ T cell purification, a TSLP stimulation time of about 20 min, sample preparartion for flow cytometry analysis (fixation, permeabilization, staining), and flow cytometric analysis. In this exemplary embodiment, $CD4^+$ T cells are isolated from whole blood, stimulated in vitro with TSLP, followed by fixing and permeabilization of the cells and detection of pSTAT5 using fluorescently-labeled anti-pSTAT5 monoclonal antibodies by flow cytometry. The percentage of pSTAT5 positive $CD4^+$ T cells is determined and compared to a previously determined diagnostic cutoff in order to classify the sample a belonging to a group defined by disease status, e.g., a normal healthy subject, an EoE patient in remission, or an patient with active EoE.

EQUIVALENTS

[0075] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[0076] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[0077] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

1. A method for detecting thymic stromal lymphopoietin (TSLP) responsive cells in human blood, the method comprising

contacting cells in vitro with TSLP, wherein the cells are previously isolated from a sample of whole blood obtained from a human subject in need of treatment for an eosinophilic gastrointestinal disorder (EGID), and

detecting one or more analytes in the cells or secreted by the cells, the one or more analytes selected from phosphorylated signal transducer and activator of transcription 5 (pSTAT5), interleukin-5 (IL-5), and interleukin-13 (IL-13),

wherein the detection of the one or more of the analytes indicates TSLP responsive cells in the blood of the human subject.

2. The method of claim **1**, wherein the detection of the one or more analytes is determined to be positive or negative based upon a predetermined threshold.

3. The method of claim **3**, wherein a positive detection of the one or more analytes indicates TSLP responsive cells in the blood of the human subject.

4. The method of claim **1**, for use in a method for diagnosing or monitoring EGID in a human subject in need thereof.

5. The method of claim **4**, further comprising determining an amount of the one or more analytes and determining whether the amount is above or below a diagnostic threshold, wherein an amount above the diagnostic threshold indicates an EGID status of active disease in a method for diagnosing, or disease progression in a method of monitoring EGID.

6. The method of claim **1**, wherein the cells are selected from peripheral blood mononuclear cells (PBMC), CD4+ T cells, and memory CD4+ T cells.

7. The method of claim **1**, wherein the method further comprises a step of isolating a fraction of cells from the whole blood enriched for PBMC, CD4+ T cells, or memory CD4+ T cells.

8. The method of claim **1**, wherein the analyte is pSTAT5.

9. The method of claim **1**, wherein the analyte is IL-5 or IL-13 gene or protein expression.

10. The method of claim **8**, wherein the pSTAT5 is detected by a method comprising flow cytometry.

11. The method of claim **9**, wherein the IL-5 or IL-13 gene expression is detected by a method comprising a polymerase chain reaction (PCR), flow cytometry, or a chromatographic technique.

12. The method of claim **9**, wherein the IL-5 or IL-13 protein expression is detected by a method comprising one or more of flow cytometry, immunoassay, and a chromatographic technique.

13. The method of claim **1**, wherein the EGID is eosinophilic esophagitis (EoE) or eosinophilic gastritis (EG).

14. The method of claim **1**, wherein the subject in need is characterized as presenting with one or more clinical features selected from dysphagia, food impaction, vomiting, abdominal pain, refractory reflux symptoms, failure to thrive in young children, a diagnosis of an atopic allergic disorder, and a family member having an EGID diagnosis.

15. The method of claim **14**, wherein the atopic allergic disorder is selected from food allergy, asthma, atopic dermatitis, allergic rhinitis and allergic conjunctivitis.

16. The method of claim **1**, further comprising a step of administering an EGID therapy to the subject in need.

17. The method of claim **16**, wherein the EGID therapy is selected from proton pump inhibitor therapy, dietary therapy, anti-cytokine therapy, anti-ALOX15 therapy, anti-TSLP therapy, anti-eosinophil therapy, glucocorticoid therapy, and esophageal dilation.

18. The method of claim **17**, wherein the EGID therapy comprises anti-TSLP therapy.

19. The method of claim **18**, wherein the anti-TSLP therapy is an immunotherapy.

20. The method of claim **19**, wherein the anti-TSLP therapy comprises anti-TSLP monoclonal antibody therapy.

21. A rapid assay for detecting a thymic stromal lymphopoietin (TSLP) responsive population of cells in human blood, the method comprising

contacting cells in vitro with TSLP, wherein the cells are previously isolated from a sample of whole blood obtained from a human subject in need of treatment for an eosinophilic gastrointestinal disorder (EGID), and

detecting phosphorylated signal transducer and activator of transcription 5 (pSTAT5) in the cells,

wherein the presence of pSTAT5 indicates a TSLP responsive population of cells in the blood of the human subject.

22. The rapid assay of claim **21**, wherein the assay is performed by a method comprising flow cytometric analysis of pSTAT5.

23. The rapid assay of claim **21** or **22**, wherein the cells are selected from PBMC, CD4+ T cells, or memory CD4+ T cells.

24. The rapid assay of claim **23**, wherein the method further comprises isolating PBMC, CD4+ T cells, or memory CD4+ T cells from the sample of whole blood.

25. The rapid assay of claim **23**, wherein the assay is performed in about 2-4 hours.

* * * * *